

## Genetics of osteal plate polymorphism and microevolution of threespine stickleback (*Gasterosteus aculeatus* L.)

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**Summary.** The results of genetical studies of osteal plate polymorphism of threespine stickleback *Gasterosteus aculeatus* reported to date (Munzing 1959; Hagen and Gilbertson 1973; Avise 1976) are discussed and proposed inheritance models are considered. Results of crosses between the morphs of *G. aculeatus* from the White Sea are presented. An attempt has been made by the author to devise a unifying model for the inheritance of plate morphs within the whole range of *G. aculeatus*. The role of polymorphism in the microevolutionary processes taking place within this complex species is discussed.

**Key words:** Osteal plate – Microevolution – *Gasterosteus aculeatus* L.

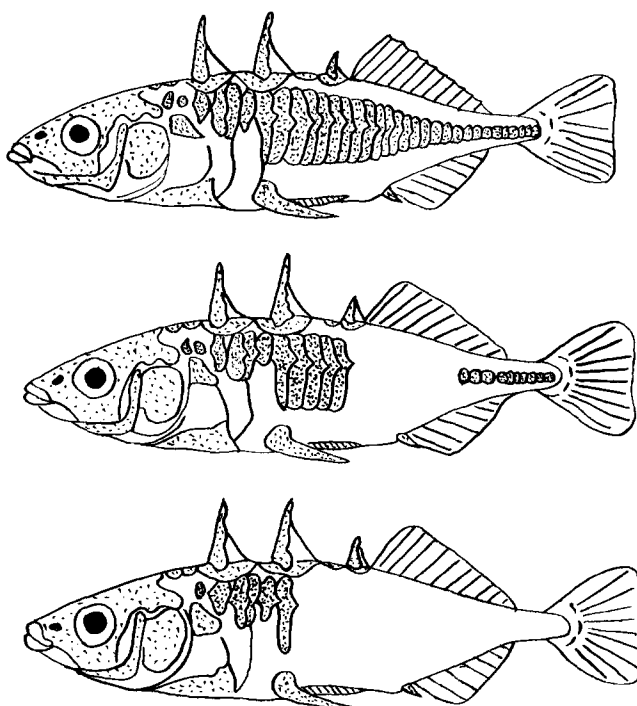
### Introduction

The threespine stickleback *Gasterosteus aculeatus* is common in coastal marine and fresh waters of the Northern hemisphere. In this species several ecological forms (marine and freshwater ones) and morphs are distinguished (Fig. 1). In correspondence with the number of lateral plates on the sides of the body three main morphs are distinguished (Bertin 1925; Munzing 1959; Bell 1976): 1) the completely plated morph = *trachurus*, with a continuous series of plates (20–30 plates, usually 25–27) covering in a tile-like manner the whole body to fuse with a caudal keel (a group of a pointed plates at the caudal peduncle); 2) the partially plated morph = *semiarmatus*, with 10–20 plates, the series of plates covering from half to three fourths of the body length is always separated from the keel by a plateless gap; 3) the few plated morph = *leiurus*, having 2–10 anterior plates (more often 5–7) and usually to keel. These three main

phenotypes are recognized by European and American authors.

In some populations, however, there occur fish with 2–9 plates (mode 5–7) as in typical *leiurus* but having also a keel. To distinguish such fish from *semiarmatus* we refer to them as “*leiurus* with a keel”, in contrast to the other authors who regard them *semiarmatus* (= partial) (Munzing 1962; Hagen and Gilbertson 1973).

The marine (anadromous) stickleback is represented by *trachurus*, while the freshwater (residential) stickle-



**Fig. 1.** The three plate morphs typically found in populations of *Gasterosteus aculeatus*. From top to bottom: *trachurus*, *semiarmatus* and *leiurus*.

back in most of the isolated basins is represented by monomorphic populations of *leiurus* or, rarely, by trimorphic or dimorphic (*leiurus* + *trachurus*, *leiurus* + *semiarmatus*) populations. In some basins of Europe and North America the freshwater form is represented by monomorphic populations of *trachurus* or (very rarely) by monomorphic populations of *semiarmatus* (*leiurus* with keel according to our terminology).

It is held by Hagen (1967) and Hagen and McPhail (1970) that the marine anadromous form *trachurus* is a distinct species from all the morphs of the freshwater form on the Pacific Coast of North America; they attribute polymorphism of the continental freshwater geographically isolated populations to natural selection rather than to introgression from the marine populations.

There have been many attempts made at analysing the genetics of polymorphism of threespine sticklebacks by several investigators who worked with different populations in various parts of the species range. In this paper the results of genetical studies reported to date are discussed and proposed inheritance models are considered. The author also presents his own results of crosses between the morphs of threespine stickleback from the White Sea. An attempt has been made by the author to devise a unifying model for the inheritance of plate morphs within the whole range of *G. aculeatus*. Also, the role of polymorphism in the microevolutionary processes taking place within the whole of this complex species is discussed.

#### Analysis of the proposed inheritance models

Munzing (1959) who worked with a polymorphic freshwater population from the Elbe (Europe) offered a monogenic two-allele scheme: AA – *trachurus* (*t*), Aa – *semiarmatus* (*s*), aa – *leiurus* (*l*), with incomplete dominance of A.

Hagen and Gilbertson (1973) on the basis of data obtained in work with a polymorphic freshwater population from the continental lake Wapato (Washington State, USA) proposed a model with two unlinked loci, with additive interaction between them, each locus with two alleles (Fig. 2).

Avisé (1976) used in his crosses the individuals from the river population Friant (California, USA), dimorphic with low and complete morphs. The segregations obtained in F<sub>1</sub> are explained by a monogenic scheme with complete dominance: "complete" – AA = Aa, "low" – aa.

One gets the impression that the same plate morph variants are controlled differently in different populations of *G. aculeatus*: monogenically with incomplete dominance in Europe, monogenically with complete

|    | AA       | Aa       | aa      |
|----|----------|----------|---------|
| BB | complete | complete | partial |
| Bb | complete | partial  | low     |
| bb | partial  | low      | low     |

Fig. 2. Inheritance model proposed by Hagen and Gilbertson (1973). The two loci are represented as *A* and *B*

dominance in California, and digenically in the populations of Washington State.

Therefore we shall now consider in detail how the segregation data obtained in the crosses performed by the named authors fit their own and others models.

Of nine possible combinations of crosses Munzing (1959) performed eight combinations (the cross ♀ *trachurus* × ♂ *semiarmatus* was not made) and proposed a monogenic model with incomplete dominance. The crosses ♀s × ♂s – 11:2s:1tr, ♀tr × ♂tr – tr, ♀l × ♂l – l, ♀s × ♂l – 1s:1l, ♀l × ♂s – 1s:1l (Munzing 1959; Fig. 10, crosses number K, L, M, A, B, N, P, Q, S), yielded segregations quite compatible with the model. However, some of his data cannot be explained by this model:

| Crosses                 | Observed segregation | Expected segregation |
|-------------------------|----------------------|----------------------|
| No. "H" – ♀s × ♂tr      | 11:2s:1tr            | 1s:1tr               |
| No. "D", "F" – ♀l × ♂tr | 2s:1tr               | s                    |
| No. "G" – ♀tr × ♂l      | tr                   | s                    |
| No. "R" – ♀tr × ♂l      | 1tr:1l               | s                    |

(Munzing 1959; Fig. 10). For these cases a digenic scheme would seem more likely. Munzing explained these results by an interference of small modifying genes (not included in the model). This explanation, however, does not seem convincing, since in cross No. "H" the modifiers change *semiarmatus* into *leiurus*, in the crosses Nos. "D", "F", "G" they change *semiarmatus* into *trachurus*, and in the cross "R" one half of *semiarmatus* is transformed into *leiurus* and the other half into *trachurus*.

So only five segregation types in his crosses strictly conform to the model, while four types cannot be accounted for by it. Besides, there are six segregation types in the crosses of Hagen and Gilbertson (1973): (tr × l – tr, l × tr – tr, l × s – l:s:tr, s × l – l:s:tr, tr × l – 11:2s:1tr, tr × l – 11:1s), and four F<sub>1</sub> segregations in the crosses made by Avisé (1976): (tr × l – tr:l, l × tr – tr:l, tr × l – tr, tr × tr – tr:l) that cannot be explained by this model either.

One cannot reject a model only on the basis of non-segregation of some theoretically expected phenotype,

especially when the numbers of offspring are small (in some of the crosses the  $F_1$  offspring is represented by only a few individuals, though normally there are 100 to 150 eggs in a clutch). The appearance of unexpected phenotypes is a far more convincing evidence for the inadequacy of the model.

Hagen and Gilbertson (1973) performed seven of nine possible crosses (combinations  $\text{♀tr} \times \text{♂s}$  and  $\text{♀s} \times \text{♂tr}$  were not made). Some of their crosses ( $\text{tr} \times \text{tr-tr}$ ,  $\text{tr} \times \text{l-s}$ ,  $\text{l} \times \text{l-l}$ ,  $\text{s} \times \text{s-tr:s:l}$ ,  $\text{l} \times \text{s-l:s}$ ,  $\text{s} \times \text{l-l:s}$ ) fit the monogenic model of Munzing, but most of them show a dihybrid segregation pattern (Nos. 29–45  $\text{l} \times \text{s-l:s:tr}$ ,  $\text{s} \times \text{l-l:s:tr}$ ; No. 46  $\text{tr} \times \text{l-l:s}$ ; No. 47  $\text{tr} \times \text{l-l:s:tr}$ ). The model of Hagen and Gilbertson is based on the segregations obtained in crosses Nos. 20–22 ( $\text{l} \times \text{l-l:s}$ ) and on the absence of segregation in the crosses Nos. 7–10 ( $\text{s} \times \text{s-s}$ ). Their model gives a more adequate fit to the segregations obtained in their crosses, though it does not account for the results of crosses No. 15 ( $\text{tr} \times \text{l-tr}$ ;  $n = 42$ ) and No. 16 ( $\text{l} \times \text{tr-tr}$ ;  $n = 39$ ). Here again the results are explained by the interference of modifiers. It should be noted that similar crosses made by Munzing (“G”:  $\text{tr} \times \text{l-tr}$ , “R”:  $\text{tr} \times \text{l-l:tr:l}$ ) are also inconsistent with their model.

Interesting results were reported by Avise (1976) who worked with an unusual population of sticklebacks (the “Friant population”) consisting only of two morphs (low and high (= complete)). He made all possible combinations of crosses (four). The results of all crosses within the population (Nos. 1–12  $\text{l} \times \text{l-l}$ , Nos. 13–23  $\text{h} \times \text{l-h:l}$ , Nos. 24–27  $\text{l} \times \text{h-h:l}$ , Nos. 28–31  $\text{h} \times \text{h-h}$ , No. 32  $\text{h} \times \text{h-h:l}$ ) are fully compatible with a single locus, two allele model with complete dominance (high –  $\text{AA} = \text{Aa}$ , low –  $\text{aa}$ ). The segregations produced in crosses Nos. 13–27 and No. 32 cannot be accounted for by the model of Hagen and Gilbertson. Somewhat different results were produced in crosses between fish from the Friant population and those from two distant populations Napa and Navarro. These crosses yielded, besides high and low, also partially plated progeny, implying a dihybrid segregation pattern: Friant ( $\text{h}$ )  $\times$  Napa ( $\text{l-h:p:l}$ ), Friant ( $\text{l}$ )  $\times$  Navarro ( $\text{h-h:p:l}$ ).

Avise (1976) offers two alternatives to explain the inconsistency of his results with the model of Hagen-Gilbertson. One is that new alleles with different dominance relationships have evolved within the Friant population; another explanation is that in this population an unusual genotypic environment was formed with the genes coadapted to favour production of only two morphs.

We have analysed all these contradictory results and suggested our own model of inheritance to account for the difference of dominant-recessive relationships in different populations of *G. aculeatus* (Ziuganov 1979). This model offers a specific mechanism of epistatic interactions between nonallelic loci, the mechanism controlling the dominance relationships in various populations of sticklebacks. In constructing the model we proceeded mainly from the fact that the crosses  $\text{tr} \times \text{l}$  and  $\text{l} \times \text{tr}$  show different degrees of dominance in different populations (complete or partial dominance). The model is based on the following assumptions. Over the whole range of *G. aculeatus* polymorphism of bony plates is determined principally by the same loci and alleles, with the same mechanism of interaction between the loci; the difference in the segregation patterns observed in the crosses between morphs is due to different

|    | CC          | Cc          | cc        |
|----|-------------|-------------|-----------|
| AA | trachurus   | trachurus   | trachurus |
| Aa | semiarmatus | semiarmatus | trachurus |
| aa | leiurus     | leiurus     | leiurus   |

Fig. 3. The relationships between genotypes and phenotypes for the three plate morphs of sticklebacks. The two loci are represented as *A* and *C*

concentrations of alleles of a regulator gene (modifier = suppressor) ranging from 1 to 0 (Fig. 3).

The relationships between the alleles “A” and “a” of the major morphogenic locus *Aa* which determines the plate morphology are controlled by a nonallelic unlinked locus we refer to as *Cc*. The latter is only indirectly involved in the development of the character in question. Its recessive allele, when in a double does, modifies the dominance of “A” from partial to complete; the dominant allele does not affect the dominance of “A” either in a homozygous or heterozygous state (genotypes  $\text{AaCC}$ ,  $\text{AaCc}$ ). The nonallelic genes “A” and “c” are epistatically related in this model. In terms of our model the populations of the Friant type which have only “high” and “low” phenotypes can be thought of as having a fixed allele “c” in a concentration of 1; in any of the crosses  $\text{h} \times \text{l}$ ,  $\text{l} \times \text{h}$  “A” will show complete dominance. There was no such fixation in the populations with all three phenotypes and genotypes “--cc”, “--Cc”, “CC”; here a dihybrid segregation pattern is to be expected. This is just what was observed in the crosses of Hagen and Gilbertson (1973), in some of the crosses by Munzing (1959), and in the interpopulation crosses of Avise (1976).

It only remains to say that all the reported segregations obtained by the named authors, with but two exceptions, are compatible with our model. The analysis of these exceptions will be given later on in this paper. First we shall present the results of our own experiments.

## Materials and methods

Unlike the other authors who worked almost exclusively with the freshwater form, we used both the marine form *trachurus* from the White Sea and the fresh water *leiurus* from the lakes of the White Sea basin. First we made the group crosses in the small isolated freshwater basins (abandoned mice quarries flooded with ground water) where no sticklebacks were found before experiment. There is a great of this kind of basin in the neighbourhood of the White Sea Biological Station (cape Kar-tesh). In 1978 five group crosses were made (Table 1).

**Table 1.** Segregation of plate morph progeny from 5 group crosses (in small isolated freshwater basins) between parents from monomorphic populations (*leiurus* – lake Mashinnoye, *trachurus* – the White Sea). tr, l and s refer to *trachurus*, *leiurus* and *semiarmatus*, respectively.

| F <sub>1</sub> brood no. | Basins (quarries) | Parents |        | Progeny |       |
|--------------------------|-------------------|---------|--------|---------|-------|
|                          |                   | No. ♀♀  | No. ♂♂ | No.     | Morph |
| 1                        | Rudnik            | 20 tr   | 20 tr  | 7       | tr    |
|                          |                   |         |        | 8       | l     |
|                          |                   |         |        | 2       | s     |
| 2                        | Chyorny           | 10 tr   | 10 tr  | 50      | tr    |
|                          |                   |         |        | 6       | s     |
| 3                        | Popov-chyorny     | 10 tr   | 10 l   | 15      | tr    |
|                          |                   |         |        | 2       | l     |
|                          |                   |         |        | 14      | s     |
| 4                        | Goluboy           | 10 tr   | 10 tr  | 38      | tr    |
|                          |                   |         |        | 29      | l     |
|                          |                   |         |        | 56      | s     |
| 5                        | Olenchic          | 10 l    | 10 l   | 31      | l     |

## Results

The analysis of the results has shown that 1) the marine form *trachurus* and the freshwater *leiurus* are conspecific and 2) the individuals of the marine monomorphic population of *trachurus*, when transferred to a freshwater basin, are able to segregate *leiurus* and *semiarmatus* as early as in the first generation (Ziuganov 1979) which favours indirectly our scheme of inheritance.

In 1979 nine individual crosses (all possible combinations) were made in similar basins, F<sub>1</sub> progeny were found in four cases (Table 2). In the other five basins no progeny were found. F<sub>1</sub> fish were caught at the stage of fry and reared in aquaria until they reached the size of adult fish (45–50 mm). Then phenotypes were determined.

The results of segregation in F<sub>1</sub> cannot be explained by a monogenic scheme (in crosses Nos. 1 and 2; Table 2) pointing rather to a dihybrid pattern of inheritance. These results fit both the model of Hagen and Gilbertson and our model. According to the first model (Fig. 2) the *trachurus* parents can only be AABB, and the *leiurus* parents either aaBb, or Aabb (in both crosses); only in this case can *trachurus* and *semiarmatus* be produced in the F<sub>1</sub> in a ratio 1:1. Consequently, all the *trachurus* offspring must be either AaBB or AABb, and the crosses between them can be expected to yield *semiarmatus* and *trachurus* in a ratio of 3:1. However, from our scheme (Fig. 3), the *trachurus* parents can only be AACc, and the *leiurus* parents (in both crosses) either aacc, or aaCc. Only in this case can *trachurus* and *semiarmatus* be produced in the F<sub>1</sub> (in a ratio 1:1 or 1:3). In the F<sub>2</sub> from the crosses between *trachurus* one waved

expect only *trachurus* and *leiurus* in a ratio 3:1. Segregation in the F<sub>2</sub> is therefore of crucial importance for deciding between the two models.

Five experiments were conducted to obtain F<sub>2</sub>. Three of them were carried out by the method of artificial fertilization in petri dishes with the young reared in aquaria. Two experiments were run in the quarries, and the young were caught 1.5 months after the beginning of the experiment (length 41–46 mm). All the crosses proved successful, the young were reared up to the adult stage and analysed for the plate morphology (Table 3). The data from crosses a, b, d, e are incompatible with the model of Hagen and Gilbertson but support our scheme. The segregation in cross “c” fits both models.

**Table 2.** Segregation of plate morph progeny from 4 individual crosses (in small isolated freshwater basins) between parents from lake Mashinnoye (*leiurus*), from the White Sea (marine *trachurus*) and from the quarry “Chyorny” (*semiarmatus*). tr, l and s refer to *trachurus*, *leiurus* and *semiarmatus*, respectively

| F <sub>1</sub> brood no. | Basins (quarries) | Parent |    | Progeny |       |
|--------------------------|-------------------|--------|----|---------|-------|
|                          |                   | ♀      | ♂  | No.     | Morph |
| 1                        | Malysh            | tr     | l  | 25      | tr    |
|                          |                   |        |    | 21      | s     |
| 2                        | Microolenchic     | l      | tr | 13      | tr    |
|                          |                   |        |    | 16      | s     |
| 3                        | Krovavy           | tr     | tr | 28      | tr    |
| 4                        | Domovoy           | s      | l  | 14      | s     |
|                          |                   |        |    | 13      | l     |

**Table 3.** Segregation of plate morph in F<sub>2</sub> progeny obtained with parents from crosses Nos 1 and 2 (Table 2). tr, l and s refer to *trachurus*, *leiurus* and *semiarmatus*, respectively. Localities shown in parenthesis: Ma = quarry “Malysh”; Mi = quarry “Microolenchic”

| F <sub>2</sub> brood no. | Parents and provenance |        | Progeny |       |
|--------------------------|------------------------|--------|---------|-------|
|                          | ♀                      | ♂      | No.     | Morph |
| a)                       | tr(Ma)                 | tr(Ma) | 31      | tr    |
|                          |                        |        | 8       | l     |
| b)                       | tr(Mi)                 | tr(Mi) | 26      | tr    |
|                          |                        |        | 10      | l     |
| c)                       | s(Ma)                  | s(Ma)  | 11      | tr    |
|                          |                        |        | 9       | s     |
|                          |                        |        | 5       | l     |
| d)                       | tr(Ma)                 | tr(Ma) | 25      | tr    |
|                          |                        |        | 6       | l     |
| e)                       | tr(Mi)                 | tr(Mi) | 21      | tr    |
|                          |                        |        | 8       | l     |

A special cross was made to find out whether it was possible for *leiurus* to be heterozygous for the morphogenic locus A. According to our scheme this morph is always homozygous for the recessive allele "a", while Hagen and Gilbertson maintain that lows can be heterozygous for both "a" and "b", and crosses between such heterozygous *leiurus* should be expected to yield 1/4 of *semiarmatus* in F<sub>1</sub>. From our model, no *semiarmatus* can be expected from such crosses.

The heterozygous *leiurus* parents in this cross were the adult F<sub>1</sub> offspring from the group cross made in the quarry "Popoy-Chyorny" (Table 1). Using the method of artificial fertilization we made a cross *leiurus* × *leiurus-leiurus* (n = 47) which yielded no *semiarmatus* (length 39–44 mm). The results may be taken as convincing evidence in favour of homozygosity of *leiurus* for the major morphogenic locus, especially as the number of offspring was large enough to exclude random death of *semiarmatus*.

It should be noted that having at our disposal only the second generation of completely homozygous *leiurus* we were unable to make the analysing crosses, i.e. the crosses between each of the three phenotypes and *leiurus* homozygous for the recessive alleles of both loci. To meet the high standards of genetic analysis we shall have to obtain the third generation. Yet, it seems that our results of the analysis of the White Sea sticklebacks prove convincingly enough the inadequacy of the model of Hagen and Gilbertson and support our scheme.

#### Analysis of segregation inconsistent with our scheme

As has been already mentioned, our scheme cannot account for the result of two types of crosses made by Hagen and Gilbertson (1973). Three crosses partial × partial gave in F<sub>1</sub> only partially plated progeny (182 individuals). The parents for these crosses were taken from the monomorphic population of partials (lake Quinault). The authors explain the nonsegregation phenomenon by homozygosity of the sticklebacks' genotypes (AA<sub>bb</sub> or aaBB). To our mind these *partials* are in fact what we call "*leiurus* with a keel".

It should be remembered here that unlike Hagen and Gilbertson who rank among lows and individuals with a low number of plates but no keel, we define *leiurus* as individuals with a small number of plates (2–10, mode 5–7) no matter whether they have a keel or not. On the other hand, any individual having a keel and bony plates on the body sides divided by a plateless gap is regarded by Hagen and Gilbertson as partial irrespective of the plate number, which means that the fish with a keel and a small number of plates (2–10) typical of *leiurus* (low) may be also placed with partials. In our classification partials (*semiarmatus*) are individ-

uals which have a keel and a great number of plates (10–20) with a gap between.

Most of populations of *leiurus* consist of low-plated fish without a keel, though there are occasional populations of low-plated sticklebacks with a keel. We regard them as homozygous "*leiurus* with a keel" rather than homozygous partials (= *semiarmatus*), since typical *semiarmatus* have usually 10 to 20 plates. The analysis of plate morphology of sticklebacks from monomorphic populations of partials from lakes Izneek (Munzing 1962) and Quinault (Hagen and Gilbertson 1972) and some others has shown that these fish have the small number of plates characteristic for *leiurus* (3–9, mode 5–7). We consider such populations as monomorphic for homozygous "*leiurus* with a keel". From this viewpoint the existence of monomorphic populations of partials should not puzzle us any more.

Thus, we believe that the three phenotypes of stickleback are really phenocomplexes with the keel at the caudal peduncle and the plates on the body sides being different independent characters determined by different loci and not alternatives of a single character, as seems to be the idea of other authors. Our model accounts only for the loci determining the plates leaving out the keel.

We assume that the dominant allele of the morphogenic locus "A" which is responsible for the great number of plates is linked with the dominant allele of the locus determining the keel (phenotype *trachurus* = complete); the recessive allele "a" is assumed to be linked with the allele responsible for the absence of the keel (*leiurus* = low); an individual heterozygous for both the gene determining the keel and that determining the plate number has phenotype *semiarmatus* = partial. In the populations of "*leiurus* with a keel" crossingover must have occurred, so that the recessive allele "a" became linked with the dominant allele determining the keel. The result of crossingover being fixed, no segregation for *trachurus*, *semiarmatus* and *leiurus* takes place in the crosses between "*leiurus* with a keel". On the other hand, crosses between partials (offspring from the crosses complete × low) will always give all three morphs, and this is just what was observed in the crosses of Hagen and Gilbertson (1973) and in our own experiments.

This view is supported by a discovery of a rather rare phenotype "*semiarmatus* without a keel" (14–17 plates) in some populations of the White Sea basin (our unpublished data) and by the existence of phenotype "*trachurus* without a keel" in the related species *Pungitius platygaster* (Munzing 1969). From the above reasoning it may be concluded that neither the existence of monomorphic populations of partials, nor the absence of segregation in the crosses between "*leiurus* with a keel" are in conflict with our scheme.

Three low  $\times$  low crosses of Hagen and Gilbertson (1973) produced in  $F_1$  altogether 43 lows and 8 partials. This segregation does not fit our model. However, other 27 crosses low  $\times$  low made with fish from the same population of lake Wapato gave no partials. Thus, 30 low  $\times$  low crosses produced 1071 offsprings of which 8 were partials (0.0074 of the total). Numerous *leiurus*  $\times$  *leiurus* crosses by Munzing (1959), Lindsey (1962) and Avise (1976) never produced any *semiarmatus*; nor were *semiarmatus* produced in the special *leiurus*  $\times$  *leiurus* cross (see above). These results are us no grounds for giving up the idea of complete homozygosity of *leiurus* for the recessive allele of the major morphogenic locus, nor does it seem reasonable to revise our model of inheritance as yet.

It should be stressed that the scheme advanced here concerns only the genetics of lateral plates. As for the keel, this character needs a scheme of its own. And to develop such a scheme would require genetic data from the crosses involving, besides the three main morphs, the phenotype "*leiurus* with a keel". Of course, our scheme is not the only possible one, and it will most likely be modified as new data emerge.

#### *Microevolution of sticklebacks in the basin of the White Sea*

From the works of Mukhomedyarov (1966) and Potapova (1972) the marine population of sticklebacks was known to consist of the *trachurus* morph with a small admixture of *semiarmatus* (0.5–1.0%). Later, monomorphic populations of *leiurus* were found in the glacial freshwater land-locked lakes on the coast (Ziuganov 1978). These lakes, mainly insular, coastal, and no older than 5–7 thousand years, were formed as a result of upheaval of the White Sea shield followed by sea regression and filling with fresh water. Therefore, the freshwater form *leiurus* must have evolved from marine *trachurus* over a period no longer than 5–7 thousand years. This is an interesting fact, as we know that splitting of *G. aculeatus* into *leiurus* and *trachurus* had taken place as long ago as 10 million years, in the Miocene (fossils of *trachurus* and *leiurus* were found respectively in marine and alluvial deposits (Bell 1976)); besides, on the Pacific coast of North America *leiurus* and *trachurus* are separate species (Hagen and McPhail 1970). Consequently, the two morphs, *trachurus* and *leiurus* could not have evolved once, by divergence, to expand over the present-day range independently of each other. It would seem more likely that in different basins populations of *leiurus* may evolve from *trachurus* in parallel.

There was much controversy in the literature over the question whether polymorphism of freshwater populations of sticklebacks is the result of secondary intergradation between the marine (anadromous) from

*trachurus* and freshwater *leiurus* or it is the outcome of selection (Miller and Hubbs 1969; Hagen 1967). The views held were mutually exclusive.

We have found two freshwater trimorphic (low + partial + complete) populations of sticklebacks in lakes Klyukvennoye and Martsy (Veliky island, White Sea). Fish of the marine *trachurus* migrate into these lakes to breed. These marine sticklebacks are marked with marine parasites (class Nematoda), while the freshwater inhabitants of these lakes are infected with a freshwater parasite *Schistocephalus solidus* (class Cestoda). Clearly in this case polymorphism is due to introgression of the *trachurus* genes into the freshwater population because neighbouring isolated lakes (Svyatoye, Lobaneshskoye) are inhabited only by monomorphic populations of *leiurus*.

Not far from these lakes we found an isolated freshwater basin occupied by a trimorphic population of sticklebacks (Ziuganov 1978). This basin was formed in the 1940's and, as we learnt from the local fishermen, several dozens of *trachurus* from the sea were introduced into it 17 years ago. In this population polymorphism must be the result of segregation of *leiurus* and *semiarmatus* from *trachurus* and of absence of selection against *leiurus* and *semiarmatus* in the fresh water. Earlier in the paper we have described two experiments with group tr  $\times$  tr crosses (Table 1) made in freshwater quarries. These experiments unambiguously confirmed the fact of segregation of *leiurus* and *semiarmatus* as early as in the  $F_1$ .

It seems we have now enough reason to speak of two different sources of polymorphism in freshwater populations: 1) secondary intergradation and 2) change of direction of natural selection as a result of a change of habitat (from sea water to fresh water).

As no definite function is ascribed to this character (lateral bony plates), it is considered merely as a marker which is probably related, pleiotropically or in some other way, to the important components of the organism fitness. Thus, the marine monomorphic population of *trachurus* is satiated with recessive alleles of morphogenic locus "Aa" in the heterozygous state. We do not know, as yet, the frequency of allele "a" and, besides, it may change with the year and from region to region. Yet, we think it to be high enough since twenty to forty founders (*trachurus*) segregate *leiurus* and *semiarmatus* in  $F_1$  when placed in the freshwater basin. In the White Sea a certain degree of heterozygosity of the population of sticklebacks is probably maintained by frequent migrations of the *leiurus* young (we observed such migrations from the lakes to the sea), and also, probably, by an increased fitness of individuals heterozygous for "A" genotype "Aacc" (*trachurus*). At the same time the selection pressure in the sea seems to be directed against *leiurus* and *semiarmatus*, that is genotypes aacc, aaCc,

aaCC, AaCc, AaCC for no adult *leiurus* was ever found in the sea, and *semiarmatus* occurs with a very low frequency (0.5–1%).

When the marine *trachurus* colonizes small isolated freshwater basins the probability of passage of recessive alleles into the homozygous state sharply increases, and the *leiurus* morph segregates. This morph seems to be favoured by selection in the fresh water and a shift in the direction of selection takes place. Marine and freshwater fish are faced with diametrically opposed osmoregulatory problems: to excrete salts from the body and absorb water (in salt water) and to absorb salts and excrete water (in fresh water). One may suppose that there exists a pleiotropic relation between the lateral bony plates and the character of osmoregulation. It was shown that the upper salinity level for the activation of sperm of the marine form *trachurus* in the White Sea is 52‰, that for *leiurus* (from monomorphic populations of *leiurus* in the glacial freshwater land-locked lakes on the coast) 16‰, and in the White Sea the average salinity is 26‰ (Ziuganov and Khlebovich 1979; Ziuganov 1981a, b).

In the course of time (7 thousand years at most) the selection on the islands and on the coast of the White Sea left only the *leiurus* morph. Before a population attains monomorphism of the *leiurus* morph it must pass through a more or less lengthy stage of transitive polymorphism, a stage at which all three morphs are present. Transformation of a freshwater population into the monomorphic population of *leiurus* is an irreversible process as follows from our scheme of inheritance. However, even a small inflow of the *trachurus* genes from the sea steadily maintains polymorphism of freshwater populations. A single artificially provoked contact between *trachurus* and *leiurus* in the isolated quarries (Popovchyorny, Goluboy, Table 1) resulted in the emergence of trimorphic populations in the next generation, and the polymorphic state of populations was maintained during the four years that we made observations (our unpublished data).

The establishment of this new scheme of morph inheritance helped in finding the mechanism of appearance of morphological diversity in a new freshwater population formed at colonization by the marine form of a freshwater basin. However, we are still unable to understand the nature of microevolutionary changes taking place in the newly formed freshwater population. Knowledge of genetics of polymorphism cannot help us to explain how or why the establishment of monomorphism of the *leiurus* takes place, or what the mechanism of selection for *leiurus* in the freshwater is. To comparatively assess fitness of *leiurus* and other morphs for the freshwater conditions requires either fine physiological experiments or longterm observations on the newly formed freshwater populations.

The American authors found a close correlation between the high frequency of seven plated *leiurus* and predation (Hagen and Gilbertson 1972). In the basin of the White Sea monomorphic populations of *leiurus* (mode 7 lateral plates) were found in both of the basins with predators (*Salmo trutta*, *Esox lucius*, *Perca fluviatilis*) and without them. This makes us believe that selection for *leiurus* is controlled by abiotic factors (salinity) rather than biotic ones.

There is no doubt that in the basin of the White Sea the marine morph *trachurus* is conspecific with all three morphs of the freshwater form which is indicated by the results of experiments described here, as well as by the data from the analysis of some meristic characters and electrophoretic spectra of some proteins (Ziuganov 1978, 1979). Consequently, in this region we are observing the beginning stages of a microevolutionary process. Nevertheless, certain populations of *leiurus* in various parts of the range of *G. aculeatus* may reach the rank of subspecies, semispecies, or even that of the species with respect both to each other and to the marine *trachurus* their common ancestor (Hagen and McPhail 1970; Moodie 1972; Bell 1976).

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