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**TWO-WAY SELECTION FOR AGGRESSION IN JUVENILE,
FEMALE AND MALE STICKLEBACKS (*GASTEROSTEUS
ACULEATUS* L.), WITH SOME NOTES ON
HORMONAL FACTORS**

by

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(With 6 Figures)
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Introduction

In his study on territory in male three-spined sticklebacks VAN DEN ASSEM (1967) found a positive correlation between territory size and aggression (expressed as the number of attacks initiated by the territory owner) in a situation with several rival males. Moreover, territory size was positively correlated with courtship-success and parental-success. These findings were confirmed by LI & OWINGS (1978). When one is interested in evolutionary processes such obvious connections of territory size and aggression with fitness are bound to lead to questions whether and to what extent the variation of territory size and aggression are attributable to genetic variation.

A feasible operational approach to such questions would be to subject the character concerned to artificial, directional selection. To that aim environmental variables have to be standardized as much as possible. It is, however, more difficult to standardize measurements of territory size than those of aggression levels. In this study we therefore concentrate on

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the influence of genetic factors on the inter-individual variation of aggressive behaviour. The relationships between aggressiveness and territory size will be an issue for further research. A test to measure aggressiveness of three-spined sticklebacks under standardized circumstances has been developed by VAN IERSEL (1958) and since then applied in many studies on stickleback behaviour (see BAKKER & SEVENSTER, 1983).

Very few selection studies have been carried out with sticklebacks as experimental animals. HAGEN & BLOUW (1983) have published a bidirectional selection experiment for dorsal spine number in the four-spined stickleback, *Apeltes quadracus*. The three-spined stickleback, *Gasterosteus aculeatus* L., is also a feasible species for genetic study. As this species has been a favourite subject in ethological research during the past 50 years, its behaviour has become well-known and it is therefore cut out for behaviour genetic research. Despite it, the study of SEVENSTER & 't HART (1974) on "double creeping through" is the only published one in which behaviour genetic methods (directional selection, classical Mendelian crossing schemes) are applied to the three-spined stickleback. In the same department of ethology at Leiden two further selection studies have been carried out using behavioural variables of the three-spined stickleback as the criterion of selection. SEVENSTER & FEUTH-DE BRUIJN (unpublished results) have subjected a strain with a high percentage of "maniacs" (males which creep through two or more times in quick succession) to selection for more and for less extreme manifestations of this deviation in behaviour. As a continuation of his study on territory VAN DEN ASSEM has made an attempt to select for dominance. This pilot study gave some indications for a fast response to selection (VAN DEN ASSEM, unpublished results). The first aim of the present study is to investigate this topic in greater detail.

A difficulty that is met when one wants to select for aggressiveness is which criterion of selection to choose. Functional concepts of aggression try to encompass aggressive behaviour, even in the most divergent situations. It is, however, questionable whether the proximate factors for aggressive behaviour in different contexts are identical. HUNTINGFORD (1976) has reviewed the literature concerning the relationship between inter- and intra-specific aggression and concludes that they are neither invariably linked nor inevitably distinct motivationally. The same indistinct situation arises if only the causation of intra-specific aggression is considered (compare *e.g.* LORENZ, 1966, and MOYER, 1968). It might therefore make a difference which manifestation of intra-specific aggressiveness is taken as a criterion of selection.

The aggressive behaviour of three-spined sticklebacks, particularly that of males, has been studied in greater detail than in most other animal species (see WOOTTON, 1976, for a review). Yet in this species our knowledge of the relationships among different manifestations of intra-specific aggressiveness is still rather incomplete. The second aim of the present study is to investigate whether intra-specific aggressiveness in different situations is influenced by the same genes.

To achieve both aims of this study, intra-specific aggressiveness of three-spined sticklebacks is measured in five different test situations. These comprise not only several different tests on reproductive males, but also tests on juveniles and reproductive females. Each of the aggression scores in a number of these situations is used as a criterion of selection in two-way selection experiments. A total of six different selection lines and one, unselected control line are bred and maintained. The responses to selection for aggressiveness yield information about the extent to which the inter-individual variation of aggressiveness in each of the situations concerned is attributable to genetic differences between individuals. To realize the second aim of this study, individuals selected for their aggression score in a particular situation are also scored for their aggressiveness in the other situations. This enables us to assess whether selection for aggressiveness in a particular situation also results in changes of aggressiveness in the other test situations. From these data it can be deduced to what extent aggressiveness in different situations is influenced by the same genes.

This paper summarizes some main results and conclusions of this behaviour genetic study on aggressiveness in the three-spined stickleback. A detailed paper of this study will soon be published in this journal.

Material and methods

The base population used in this study consisted of a sample of about a hundred, randomly chosen, young from fifty wild-caught parents (Vaassen, The Netherlands), which belonged to a non-migratory population of low-plated morphs (forma *leiura*; BERTIN, 1925). The fish were kept under highly standardized conditions: the young were isolated (physically and visually) a few weeks after hatching in plastic tanks (34 × 17 × 20 cm), which were set up in a standardized way and placed in an airconditioned room (temperature 18-20°C) with long day-lengths (16L/8D). They were fed twice a day on live *Tubifex* worms, *Artemia*, *Chironomus* larvae, and frozen *Artemia* or *Mysis*.

Behavioural tests.

The aggressiveness of the fish was assessed in a number of different test situations with the aid of behavioural tests (Fig. 1). Males were submitted to juvenile, male, courtship and

dominance tests. Their aggressiveness in these test situations will be called juvenile aggressiveness, territorial aggressiveness, courtship aggressiveness and dominance ability (or dominance), respectively. Females were submitted to juvenile and female tests in order to measure their juvenile aggressiveness and female aggressiveness, respectively. In these behavioural tests, except in the dominance tests, the experimental fish was confronted in his own tank with an opponent of about the same age and size enclosed in a glass tube that was presented inside the tank or a plastic chamber that was hung outside at the front of the tank. In the tests the experimental fish was given an opportunity for 5 minutes after introduction of the opponent to reach the tube or chamber. Its behaviour was recorded for 5 minutes from then on. Juvenile tests were carried out once per week (in later generations one test each two-week interval) beginning a few weeks after isolation and ending when sexual maturity was attained. The aggression tests on adult fish (female, male and courtship tests) were carried out once a week in four successive weeks after the attainment of sexual maturity.

Fig. 1. Outline of behavioural tests performed in the study.

Behavioural tests	Experimental animal(s)	Opponent
Juvenile test	juvenile	juvenile
Female test	♀	sub-adult
Male test	♂	♂
Courtship test	♂	ripe ♀
Dominance test	♂, ♂	—

Fig. 2. Selection lines and the criteria upon which selection was based. H = high line, L = low line.

Selection line	Abbr.	Criterion of selection	
		♂♂	♀♀
Juvenile Aggression Lines	JH JL	Juvenile Aggression Score (JAS)	Juvenile Aggression Score (JAS)
Territorial Aggression Lines	TH TL	Territorial Aggression Score (TAS)	Female Aggression Score (FAS)
Dominance Lines	DH DL	Dominance Ability	Dominance Ability of their brothers
Control Line	C	—	—

Aggressiveness in the various test situations was expressed as the total duration of biting and bumping at the opponent related to the period the experimental fish stayed within a fish-length distance of the opponent in the case of juvenile and female tests (this measure will be called “% biting-bumping in the tube-period”) or to the 5 minutes test period in the case of male and courtship tests (this measure will be called “% biting-bumping in the test-period”). To compare the aggressiveness in various situations, and to select for aggressiveness in a particular situation, it is necessary to have individual aggression scores at one's disposal. These were obtained by averaging for each fish the outcomes of the various tests in a particular situation. To calculate juvenile aggression scores only test outcomes prior to week 15 after fertilization were taken into account.

After the completion of the male and courtship tests the same males were submitted to dominance tests. In these tests two, isolated, territorial males were introduced simultaneously into a tank that was unfamiliar to both, and just large enough for one male to settle a territory. The dominance test lasted until a clear-cut dominance of one male over the other was established (usually within 10 minutes after their introduction). When individuals taken from a group of isolated males were tested in all possible combinations, the large majority of test results were in agreement with a linear order of dominance of the males concerned (BAKKER & SEVENSTER, 1983). The rank of a male in the linear order will be called his dominance ability or dominance. For more details the reader is referred to BAKKER & SEVENSTER (1983).

Selection lines.

From the base population six different selection lines were founded according to the criteria presented in Fig. 2. The selection procedure was repeated four times, so that 5 generations (the base population and 4 selected generations) were involved. The first three selected generations were treated identically to the base population, that is all fish were submitted to identical rearing conditions and to an identical set of behavioural tests as in the base population. For technical reasons the frequency of the juvenile tests was, however, reduced to one test each two-week interval. The fish of the fourth generation were used in another project and not submitted to the behavioural tests outlined above. In each selection line and in each generation we aimed at breeding three parental pairs, chosen from the most or the least aggressive individuals of both sexes. In selecting potential parents, only the aggression score relevant for the selection line in question was taken into account. Extreme inbreeding was avoided in order to reduce the influence of genetic drift. In each selection line a sample of about thirty, randomly chosen young from the progeny of the parental pairs was isolated individually to form the next generation. Besides the six selection lines an unselected control line was maintained, which consisted every generation of the progeny of about twenty, randomly chosen parents. In generation 2 juveniles of the control line were again isolated (and also in generation 4) and tested. For more details and reasoning of the methods that were applied in this study the reader is referred to BAKKER (in press).

Results and discussion

Under this heading some main results and conclusions are presented of this behaviour-genetic study on aggressiveness in the three-spined stickleback. This presentation should be considered as a rather comprehensive summary of this study, so many details and side-issues are omitted, which will be extensively treated in BAKKER (in press). The following three main topics are reviewed here: the interrelations of aggressiveness in the different test situations in the base population, the results of selection for aggressiveness, and lastly some changes in characters not directly selected for.

Aggressiveness in the base population.

In each of the different test situations aggressiveness is highly variable across individuals of the base population. For each test situation individual aggression levels are averaged to facilitate comparison between

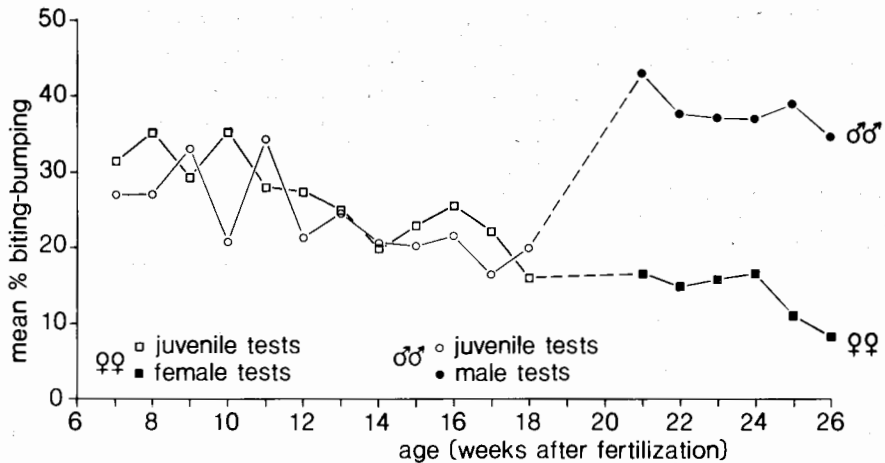


Fig. 3. Mean level of aggression as a function of age in the base population. Aggression expressed as % biting-bumping in the tube-period, except for adult males where it is related to the total test time.

test situations. In Fig. 3 these mean aggression levels are shown as a function of age for aggressiveness exhibited in the juvenile, female and male tests. It is obvious that the mean level of aggression of males and females does not differ during the juvenile phase (*cf.* SEVENSTER & GOYENS, 1975). The transition to sexual maturity in the females is not accompanied by a change in aggressiveness (*cf.* SEVENSTER & GOYENS, 1975), although the slight and persistent decline of aggression with age observed during the juvenile phase continues into the adult stage. The general decline of juvenile aggressiveness with age is not understood. It cannot be ascribed to habituation to the test situation. The mean aggressiveness of males increases considerably once they reach sexual maturity. This increase of aggressiveness coinciding with sexual maturity in males is in concordance with data from several other studies (see WOOTTON, 1976).

A comparison between the individual, mean aggression levels (the aggression scores) obtained in the different behavioural tests yields more information about the interrelations of aggressiveness in the different test situations. In females aggressiveness during the juvenile and adult stages shows a rather high phenotypic correlation coefficient, whereas the correlations between aggression scores of males are lower (Table 1). Only territorial and courtship aggressiveness are significantly correlated in males (*cf.* SEVENSTER, 1961). Dominance ability and territorial ag-

TABLE 1. Spearman rank correlation coefficients between aggression scores obtained with different behavioural tests

	Juvenile test	Male test	Courtship test
Female test	+ 0.61(33)**	—	—
Male test	+ 0.24(23) ^{ns}	1	—
Courtship test	+ 0.14(23) ^{ns}	+ 0.30(33)*	1
Dominance test	- 0.14(7) ^{ns}	+ 0.25(7) ^{ns}	+ 0.45(7) ^{ns}

() = number of fish. ** = $p < 0.01$; * = $p < 0.05$; ns = $p > 0.05$.

gressiveness are not correlated. However, the number of males upon which the correlation is based is small, since not all base population males were ranked in a dominance order. The males of the base population were randomly divided into a number of groups and the most and least dominant male in each group was determined with a series of dominance tests. Finally, these selected males were ranked in a linear order of dominance. BAKKER & SEVENSTER (1983) provide additional data that confirm the absence of a correlation between dominance ability and territorial aggressiveness.

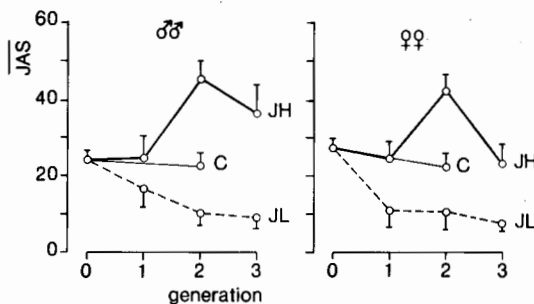


Fig. 4. Responses of the juvenile aggression lines. \bar{JAS} = mean juvenile aggression score. Lengths of bars represent one standard error of the mean.

Selection for aggressiveness.

From the base population (generation 0) six different selection lines were founded. Males and females show similar responses to two-way selection for juvenile aggressiveness (*cf.* left and right graphs in Fig. 4). Selection for a reduced juvenile aggression level has been highly successful in both sexes; even after one generation of selection a significant decrease as

compared to the base population is attained for either sex. Selection in an upward direction appears less effective. Although selection has effected a significant increase in both sexes after two generations of selection, the results of selection are cancelled out in the next generation. Several causes may be involved in this decline of juvenile aggressiveness in generation 3. This paper is an inappropriate place to discuss each of them in detail (but see BAKKER, in press). A main cause is probably limitations

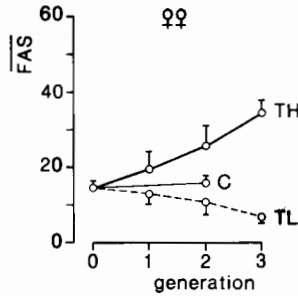


Fig. 5. Responses of females to selection for female aggressiveness. $\overline{\text{FAS}}$ = mean female aggression score. Lengths of bars represent one standard error of the mean.

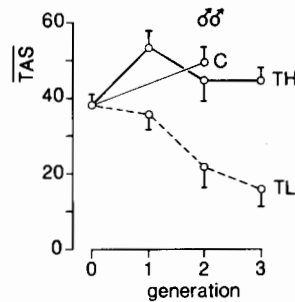


Fig. 6. Responses of males to selection for territorial aggressiveness. $\overline{\text{TAS}}$ = mean territorial aggression score. Lengths of bars represent one standard error of the mean.

of breeding the selected parents in generation 2 of the JH line. Because of extreme aggressiveness of ripe females, several attempts to breed resulted in intense roundabout fights between the sexes. Moreover, in one of the four produced clutches it is likely that selective embryonic death has occurred.

The responses to selection for female aggressiveness in opposite directions are almost symmetrical (Fig. 5). So in reproductive females ag-

gressiveness is as easily enhanced as it is lowered. Two generations of selection effects already a significant difference in mean female aggressiveness between high and low line females.

The response to bidirectional selection for territorial aggressiveness in reproductive males is, on the contrary, highly asymmetrical (Fig. 6). The high line shows no response at all, while selection in downward direction is highly successful (after two generations of selection a significant decrease as compared to the base population is attained). FALCONER (1981) enumerates eight main causes that may generate asymmetrical responses. However, none of these can satisfactorily explain the lack of response in males of the high territorial aggression line. The most plausible explanation for this lack seems therefore that the base population has already been at an upper selection limit for territorial aggressiveness.

TABLE 2. Differences between mean dominance abilities of DH, DL and C line

Dominance tests between	Number of tests	Won by DH ♂♂	
		n	%
DH-1 / DL-1	19	12	63.2
DH-2 / DL-2	9	6	66.7
DH-3 / DL-3	24	19	79.2
		Won by C ♂♂	
		n	%
DH-2 / C-2	10	5	50.0
DL-2 / C-2	10	7	70.0

The responses to selection for dominance ability cannot simply be calculated, since dominance ability is a relative measure. To determine the joint response to two-way selection for dominance ability, in each generation (after the assessment of the linear orders of dominance of both separate lines) inter-line dominance tests were held with males randomly chosen from the high and low dominance line. The deviation from 50% gain by males of the high or low line is a measure of the difference in mean dominance ability between the high and low dominance line. The discrepancy between the lines increases with the progress of selection, and in generation 3 reaches the 1% level of significance (Table 2). So selection for dominance ability has been successful in one or both directions. Confrontations of high and low line males with those of the control

line (lower part of Table 2), and with males of the other selection lines show that the increasing difference in mean dominance ability between the selection lines is due to a gradually decreasing mean dominance ability of the low line. The high line does not respond to selection for increased dominance ability. Since selection for enhanced dominance ability is accompanied by a clear reduction in viability of fertilized eggs, the lack of response in the high line probably results from mortality of "highly dominant" genotypes.

In summary, the presented results of the selection experiments clearly show that, under the applied experimental conditions, the variation of juvenile aggressiveness, female aggressiveness, territorial aggressiveness, and dominance ability can to a considerable extent be ascribed to additive genetic variation. The combined two-way responses yield heritability-estimates of at least 0.30 for aggressiveness in each of the test situations. The realized heritabilities in the single selection lines range from 0 to at least 0.60. In spite of the large genetic component under these standardized circumstances, there obviously are limitations to selection, especially in the upward directions.

Indirect selection for aggressiveness and for some other characters.

As with the phenotypic value of one character, the correlation of phenotypic values of two characters, the phenotypic correlation, may be partitioned into its genetic and environmental parts. The genetic correlation then expresses the extent to which two characters are influenced by the same genes. In selection studies the degree of genetic correlation may be deduced from the changes in characters not directly selected for as compared to the response to selection. To answer the question: "Is there a common genetic basis for aggressiveness in the different test situations?", fish of a particular selection line were not only scored for their aggressiveness in the particular situation in which they were selected, but also for their aggressiveness in the other test situations, the criteria of selection in the other selection lines. The context of this paper does not allow a detailed treatment of this item (but see BAKKER, in press). So I have to confine myself here to a descriptive summary of the results.

In both the juvenile aggression lines and the territorial aggression lines there are striking similarities between the changes of mean juvenile aggression score of juvenile females and mean female aggression score with proceeding selection. This means that many genes, if not all, affecting

aggressiveness of females in both situations are identical. The rather high phenotypic correlation between juvenile and female aggression score in the base population can in this case be translated into a high genetic correlation. Apparently, the genetic influence on aggressiveness in females does not change with sexual maturity.

In males the situation is more complicated. Aggressiveness of juvenile males, which is indiscernable from that of juvenile females, is only partly governed by the same genetic factors as territorial aggressiveness. Juvenile aggressiveness and dominance ability are genetically uncorrelated, as can be deduced from the similar, mean juvenile aggression levels in juvenile males of both dominance lines and from the similar, mean dominance abilities of males of both juvenile aggression lines. Territorial aggressiveness and dominance ability probably have a different genetic background, since selection for dominance ability is not attended by a change in territorial aggressiveness. A comparable result was obtained by FRANCIS (1984), who subjected the paradise fish (*Macropodus opercularis*) to bidirectional selection for dominance ability. Selection for territorial aggressiveness does, on the other hand, result in a significant difference between mean dominance ability of the high and low line. This difference can however be traced back to a difference in the brightness of colouration (which is an important determinant of dominance in three-spined stickleback males; BAKKER & SEVENSTER, 1983) between males of the two territorial aggression lines. Although courtship aggressiveness was not used as a criterion of selection, males of the different selection lines were nonetheless scored for their aggressiveness during courtship tests throughout the selection experiments. Changes of courtship aggressiveness parallel those of territorial aggressiveness in the various selection lines, suggesting that both these manifestations of aggressiveness have a similar genetic basis. In summary, when genetic influences are considered, it is clear that aggressiveness of three-spined stickleback males in different situations by no means are manifestations of one and the same process.

As a final item of this paper I present some changes other than aggressiveness that characteristically accompanied selection in the three different pairs of selection lines. In the juvenile aggression lines there exists a negative correlation across the generation means between juvenile aggression score and age of sexual maturity. Furthermore, high and low line fish of the juvenile aggression lines differed in age at onset of juvenile aggression, and females of the high and low juvenile aggression lines differed in the incidence of ripeness. It is therefore likely that the hormonal

causation of juvenile aggressiveness in both sexes can be ascribed to gonadotropic hormones. This is in agreement with the results of several other studies in which it was shown that the level of aggression in three-spined stickleback males before sexual maturity is associated with the level of gonadotropins (see WOOTTON, 1976).

In male three-spined sticklebacks the kidneys have been transformed from excretory into predominantly secretory organs that produce large amounts of mucus for nestbuilding. This glandular transformation, which is accompanied by about a seven-fold increase in circumference, is effected directly by androgens (DE RUITER & MEIN, 1982). Kidney size differences are therefore very likely to reflect differences in androgen levels. Kidney size measurements made on fourth generation, reproductive males of the various selection lines, reveal that only selection for territorial aggressiveness has resulted in enlarged and reduced kidneys of high and low line males, respectively. Selection for aggressiveness in the other test situations has not effected changes in kidney size. From these data it can be concluded that selection for territorial aggressiveness has most likely acted on the level of androgens, either directly or indirectly. Although no change is found in mean kidney size of low juvenile aggression line males, yet the mean level of territorial aggression is reduced in these males. This suggests that the causal factors for juvenile aggression, which likely are gonadotropic hormones, are still operating during the sexual phase (cf. WOOTTON, 1970).

In a previous publication it was shown that in the three-spined stickleback male brightness of colouration is an important determinant of dominance (BAKKER & SEVENSTER, 1983). On various occasions the colouration of the males was quantified by a four-point scale. Selection for dominance ability has brought about a difference in brightness of colouration between high and low dominance line males; after three generations of selection males of the low line are significantly duller than males of the high line and the control line. In view of the unchanged mean kidney size in the dominance lines, selection for dominance ability has probably acted on androgen-independent factors that influence the brightness of colouration.

In summary, changes other than aggressiveness accompanying selection for aggressiveness in different situations once again affirm the heterogeneous causation of aggressiveness of three-spined stickleback males exhibited in different situations.

Summary

This paper summarizes some main results and conclusions of a behaviour genetic study on intraspecific aggressiveness in the three-spined stickleback (BAKKER, in press). Bidirectional selection experiments with aggressiveness in a number of test situations each serving as a criterion of selection, reveal that the phenotypic variation of aggressiveness in juvenile and adult females, in juvenile males, of territorial aggressiveness and dominance ability in reproductive males can to a considerable extent be ascribed to genetic variation. Selection for enhanced aggressiveness generally is less effective than for reduced aggression levels. In females the genetic influence on aggressiveness in the juvenile and adult stage is most likely identical. However, in males different manifestations of aggressiveness (as measured in a number of test situations), though covered by the functional Aggression-concept, by no means represent a unity, neither with respect to their genetic causation nor with respect to their hormonal causation.

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