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AGGRESSIVENESS IN STICKLEBACKS (GASTEROSTEUS ACULEATUS L.): A BEHAVIOUR-GENETIC STUDY

by

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1. General introduction

1.1. Genes and aggression.

Aggression has been a favourite topic of scientific research since the second world-war. Since aggressive behaviour is nearly universal among animals, including human beings, there has been much speculation about its cause. Because the resulting possible explanations often conflict with the different images of man as conceived by the various religious, political and cultural groups, they are bound to lead to heated debate. In essence these debates are usually centred around the question of genetic determinism, which maintains that aggression is inherited and inevitable. Questions like "Is aggressiveness controlled by genes?" had led to the well-known "innate *versus* learned" dichotomy in the study of behaviour. This dichotomy was fed by extreme explanations of the causation of aggression such as the frustration-aggression model (DOLLARD *et al.*, 1939) or the learning theory model (BANDURA & WALTERS, 1963) on one side, and those such as the Lorenzian-Freudian drive model (LORENZ, 1966) on the other. In the sixties the evidence accumulated allowing for a synthesis of both views. It was recognized that genes are

as indispensable to behaviour as they are to *e.g.* morphological structure. This becomes evident from, for example, selection experiments with non-inbred populations of *Drosophila*: "There appears to be no character—morphogenetic, behavioral, physiological, or cytological—that cannot be selected in *Drosophila*." (LEWONTIN, 1974). However, a genetic basis for behaviour does not mean that behaviour is solely genetically determined; there is always an interaction between the genotype and the environment in the development of any biological character. So differences between individuals may be caused by either genetic differences or environmental differences or both. The question "Is aggressiveness controlled by genes?" is therefore false and should be replaced by "To what extent can the variation of aggressiveness be attributed to genotypic variation?".

The false innate-learned dichotomy of behaviour gradually seemed to disappear in the 7th decade of this century. However, with the recent interest in sociobiology (WILSON, 1975) the debate has revived. In sociobiology social behaviour is approached from an evolutionary viewpoint, and thus the genetic factor in behaviour is stongly emphasized. Moreover, this emphasis is reinforced by some of the more outrageous claims of many sociobiologists (*e.g.* WILSON, 1975, 1978) and the misleading terminology that is used to indicate genetic involvement in the expression of any character. A primary issue brought about by sociobiology revolves around the question of genetic determination of behaviour, especially where it concerns human beings. There exists a general aversion against the idea of genetic determination of behaviour, because people incorrectly think that genetic determination means something like fate, which cannot be avoided. The English sociobiologists indeed are generally more cautious when applying sociobiology to humans (*e.g.* DAWKINS, 1976; MAYNARD SMITH, 1972). But as sociobiologists they also stongly stress the role of genes in the social behaviour of animals. In the context of aggression this renewed interest in the genetic programming of behaviour has led to the "Evolutionarily Stable Strategy"-concept (*e.g.* MAYNARD SMITH & PRICE, 1973). Like all concepts in sociobiology, the ESS-concept starts from the principle that at least part of the variation in aggressiveness between individuals must be genetic in origin.

Although aggression has been a favourite topic in behavioural studies, fundamental behaviour genetic research on aggression is rather scarce. Most such studies have been concerned with the comparison of the aggressiveness of inbred strains of mice and rats. LAGERSPETZ & LAGERSPETZ (1974) and MICHARD & CARLIER (1985) summarize the results of 14, resp.

20 of such studies with mouse strains. Although the mutual comparison of these studies is difficult, due to various methodological differences, they indicate that the differences in aggression levels between mouse strains are genetically based. Similar investigations with similar results have been made with rat strains (*e.g.* HALL & KLEIN, 1942), breeds of chickens (*e.g.* FENNELL, 1945), and breeds of dogs (SCOTT, 1958; SCOTT & FULLER, 1965).

Behavior genetic methods which yield more useful information about the genetic architecture of a trait, namely assessing resemblance between relatives, crosses, and directional selection, have been scarcely applied to aggressive behaviour. The few examples that exist with regard to aggressiveness in fish (see introduction of chapter 3) confirm the picture that part of the variation of aggressive behaviour is attributable to genotypic variation. In section 3.6. directional selection experiments with aggressiveness as the criterion of selection will be reviewed and discussed in the light of the results of the present study. In all, nine selection studies have been published, five with house mice, three with domestic chickens and one with paradise fish. All but one revealed a reasonable amount of genetic variation for aggressiveness. Because of the high intensities of selection applied in those investigations, significant changes in the level of aggression are gained even after only a very limited number of generations of selection. One has to realize that such strong artificial selection pressures do often not obtain in nature. Furthermore, under natural circumstances the relevant environmental variables are far less constant than in the selection studies. Nevertheless, the few selection studies show that there is considerable genetic variation in aggressiveness present in the populations studied.

Hitherto I have used aggression in a broad, functional sense, such as "a physical act or threat of action by one individual that reduces the freedom or genetic fitness of another" (WILSON, 1975). The usual definitions of aggression encompass aggressive behaviour, even in the most divergent situations. Several authors have attempted to divide aggression into discrete categories. LORENZ (1966) categorizes aggression in terms of the three main situations in which it may occur: social (or intra-specific) aggression, predatory aggression, and anti-predator aggression. MOYER (1968) has classified aggressive behaviour in more detail and distinguishes eight categories. I will not occupy myself with the criticism of this classification, but see *e.g.* HUNTINGFORD (1976c). Although MOYER states that the categories are not mutually exclusive, he nevertheless ascribes the causation of aggression in the different categories to different

neural and endocrine factors. HUNTINGFORD (1976c) has reviewed literature concerning the relationship between inter- and intra-specific aggression and she concludes that: "The most realistic assessment of this collection of facts would seem to be that social, anti-predator and predatory aggression are neither invariably linked nor inevitably distinct motivationally.". In those species where intra- and inter-specific aggression do covary, as *e.g.* in the three-spined stickleback (HUNTINGFORD, 1976a, b, 1982), a possible common physiological basis for intra- and inter-specific aggression may have evolved under the influence of convergent selection pressures (HUNTINGFORD, 1976c).

The same indistinct situation with respect to the identity of causation of intra- and inter-specific aggression arises if only the causation of social aggression is considered. According to LORENZ's view (1966) a unitary drive underlies the social aggression shown in various contexts. The classification of MOYER (1968) further divides the "social aggression" category of LORENZ, and with that, its causation. Although data have been published supporting both views, behaviour genetic research that could help to elucidate the genetic architecture of intra-specific aggression in different situations is unfortunately almost lacking. In this respect, the present study attempts to obtain some understanding of the genetics of aggression in the three-spined stickleback. It concentrates on intra-specific aggressiveness in a number of different situations, which is quantified in most of these situations by the duration of overt aggression, *i.e.* biting and bumping. In this study, which aims at the genetic causation of aggression, no further definition of aggression (in a functional sense) is needed.

1.2. Aim of the study.

The aim of the study is two-fold:

1. To assess the extent to which the inter-individual variation of aggressiveness, as measured in each of a number of different situations, can be ascribed to genetic variation.

2. To assess the extent to which variation of aggressiveness in these different situations is influenced by common genetic factors.

In this study the intra-specific aggressiveness of three-spined sticklebacks has been measured in different situations. In each situation individual aggression scores have been obtained. To realize the first aim three of these aggression scores have been used as a criterion of selection in two-way selection experiments. Thus a total of six different selection

lines and one, unselected control line have been bred and maintained. In order to realize the second aim of this multiple selection research, individuals selected for the aggression score in a particular situation were also scored for aggressiveness in other situations.

At first sight the three-spined stickleback seems a poor choice for an experimental animal for genetic research. Its generation time is rather long (5 to 7 months), its chromosome number approaches that of man (diploid number: 42, MURAMOTO *et al.*, 1969), and inbred strains are lacking. Contrary to these restrictions, which hinder the kind of refined genetic analyses that have been carried out with *Drosphila*, there are some advantages which make the stickleback very suited for behaviour genetic research, as has been shown by a study of SEVENSTER & 't HART (1974). Since the publication of TER PELKWIJK & TINBERGEN (1937) the three-spined stickleback has been a favourite subject in ethological research and hence its behaviour has become well-known. It has an extensive behavioural repertoire, which can be relatively simply and objectively recorded. It is easily kept and bred in the laboratory under semi-natural conditions. Its way of life permits a high degree of standardization of environmental variables. Isolation, which can be carried out even from the egg stage, does not lead to any apparent behavioural abnormalities (CULLEN, 1960; SEVENSTER, 1968). Further advantages of the three-spined stickleback for behaviour genetic research are its great reproductive capacity and its well-studied ecology and endocrinology.

1.3. Aggressive behaviour of sticklebacks.

Aggressive behaviour is shown by sticklebacks in a wide variety of situations. I will confine myself here to a description of the situations in which intraspecific aggression has been measured in the present study. For a more detailed survey of the life-cycle of the three-spined stickleback, the interested reader is referred to publications of VAN IERSEL (1953), SEVENSTER (1961), and WOOTTON (1976, 1984).

In schools of juvenile sticklebacks consisting of both sexes the first signs of aggression (*juvenile aggression*) become visible at an early age, which in the laboratory is about four weeks after hatching. During the first weeks after the onset of aggression the attacks are "unilateral". The attacked juvenile flees, sometimes before a bite or bump can be delivered. The fleeing juvenile swims rapidly in a straight line away from the aggressor and stops abruptly at some distance from the latter. Most attacks are restricted to one bite or bump, unless the attacked fish fails to flee. Chas-

ing does not occur. A few weeks after the onset of juvenile aggression young will flee over a short distance immediately following an attack. But then, some time thereafter, they often turn around and counter-attack. These "bilateral" attacks may continue for some time with the attacked fish fleeing very short distances or not at all. In the latter case, roundabout fights (rapid circling with spines erect) may occur. Although territories (in the sense of defended areas) may be temporarily formed in schools of juveniles of only a few months of age, breeding territories will not be established before an age of 3-4 months (under laboratory conditions).

Young males leave the school and claim an area for nestbuilding. The ability of a male to obtain an unoccupied area in competition with other males I will call its *dominance ability*. In such situations dominance fights similar to the bilateral attacks in juveniles can be observed. Bouts of roundabout fighting are often seen in such situations and they may be intense and persistent. Very occasionally roundabout fights are interrupted by mouthfighting (BAKKER & SEVENSTER, 1983). Besides counter-attacking, the male that is attacked by a rival may flee or assume threatening displays. In the present study the latter was seldom observed during the juvenile stage.

Once a male has established a territory, he enters the nest-building phase and will defend his territory violently against intruding rival males (*territorial aggression*). The territory owner attacks the intruder with a direct charge, that ends in a bite or bump. The contest in this situation is asymmetrical and roundabout fighting seldom can be observed. Not only intruding rival males are attacked, but also intruding young, if not too small, or females and even fish of other species. However, when the nest is completed and the male enters the sexual phase ripe females are, apart from an occasional bite especially in the first moment, approached with a series of zig zags, which is part of the courtship behaviour in this species. During courtship, however, aggressive behaviour may still break through at various points in the courtship sequence (hereafter referred to as *courtship aggression*).

Adult females join other females, thus forming groups. When a female becomes ripe she may leave the school and after spawning rejoin the group of females. In these groups females also show aggressive behaviour among themselves (*female aggression*); thus aggressiveness is not restricted to the male.

Aggression can also be osberved in other contexts, *e.g.* during the parental phase, but these are not considered here.

The above outlined manifestations of aggressiveness under natural circumstances can be easily studied in the laboratory under standardized conditions with isolated fish. For the measurement of territorial aggressiveness VAN IERSEL (1958) developed an "aggression test", in which an isolated, territorial male is offered a rival male enclosed in a glass tube inside his territory. This test, which has since been applied in many studies of stickleback behaviour, has yielded useful data. In the present study this presentation technique has been extended to other situations. Dominance ability can be studied in the laboratory by introducing two territorial males simultaneously in an unfamiliar tank, which is just large enough for one male to settle a territory.

1.4. Outline of the paper.

The paper starts (chapter 2) with an analysis of the inter-individual variation of intraspecific aggressiveness in different situations within the base population, *i.e.* the population from which the selected lines were derived. There exists a considerable amount of phenotypic variation with a large genetic component for the mean individual aggression level (which I will call "aggression score" from now on) in each of the different test situations. In females the correlation between the aggression scores in different situations is rather high, but in males moderate to low. The sexes cannot be distinguished as to their juvenile aggressiveness, but in the adult stage the difference is large. In addition a number of variables with a possible influence on aggressiveness in the various test situtions are analysed: age, age at onset of sexual maturity, degree of ripeness, location of the nest, experience.

In the next chapter (chapter 3) the responses to bidirectional selection using different aggression scores as the criterion of selection are the centre of interest. For all the investigated manifestations of aggressiveness the total phenotypic variation contains a fairly large additive genetic component. Further, there are some evident restrictions to selection, particularly for enhanced aggressiveness. Some possible causes for asymmetry of response are discussed in this chapter. Selection for juvenile aggressiveness is accompanied by a shift in the onset of juvenile aggression and a shift in the age of sexual maturity of both sexes. Two-way selection for territorial aggressiveness and female aggressiveness also results in a difference of the responsiveness of the fish to the test-stimulus. Selection for dominance ability, lastly, brings about a change in the brightness of colouration of the reproductive males.

Responses to selection of characters other than those directly selected for, *i.e.* the correlated responses, are further analysed in the next chapter (chapter 4). The genetic correlations between the different aggression scores that are discussed in this chapter are comparable to the phenotypic correlations in the base population: a very high genetic correlation between aggressiveness of females in the juvenile and adult stage, and a considerably smaller one between the juvenile and adult aggression scores in males. The possible causes for asymmetry of response are discussed further. Next, a number of correlated responses, which directly or indirectly have a bearing on aggressive behaviour, are scrutinized. It is shown that females selected for lower juvenile aggressiveness also show a lower incidence of ripeness. An analysis is made of the correlated responses of aggression in other situations, such as courtship aggression and juvenile aggression in groups, and of measures of aggressiveness other than the applied criteria of selection (relative duration of biting and bumping). The chapter is concluded with an analysis of

variation in testis size and kidney size of reproductive males belonging to the different selection lines. From this it is deduced that selection for territorial aggressiveness has brought about a change in the androgen level.

Each chapter ends with a summary. Discussion of the results and relevant data from other publications are, whenever possible, presented in the sections concerned. The paper ends with a general discussion (chapter 5) and summary.

For the sake of readability several passages of this rather complicated paper have been printed in small type. In these passages some issues are discussed that are interesting in themselves (literature references, detailed discussions of exceptions and discrepancies, side-issues, and so on), but can be skipped without losing the thread and the essence of the paper. To facilitate comparison of the different responses and a number of correlated responses without looking up the figures concerned, I have inserted an appendix, which briefly summarizes the major responses and correlated responses after three generations of selection. It may facilitate reading chapter 4 and 5. For a detailed summary of the main results of this paper the reader is referred to BAKKER (1985).

2. Aggression in the base population

The base population used in this research consisted of first generation offspring from wild-caught parents and may be considered as a genetically heterogeneous population. It allows us to study:

— Inter-individual variation of aggressive behaviour within different test situations.

— Relationships between the aggression levels obtained in different test situations.

In addition, the data of the base population can be used to gain some idea of the extent to which the phenotypic variations can be ascribed to genetic causes.

2.1. Material and methods.

In the autumn of 1978 three-spined sticklebacks were caught at 13 different places in brooks of one drainage system (ultimately flowing into the Apeldoorns kanaal) near Vaassen (The Netherlands). In this way we hoped to collect a genetically heterogeneous sample of the resident population (a freshwater population which is monomorphic for the forma *leiura*; BERTIN, 1925). Because fish of about the same age and reared under the same conditions were needed for the selection experiments 25, randomly chosen pairs of the wild-caught sticklebacks were bred in the laboratory.

2.1.1. *Rearing conditions.*

From each of these 25 fry 5 randomly chosen young were isolated individually in plastic tanks of 34 x 17 x 20 cm at the first sign of aggression in the fry. The tanks were set up in a standard manner with a layer of *ca* 4 cm sand, three longleafed plants (*Vallisneria*, *Allisma*, *etc.*) and some small tufts of green, filamentous algae (Fig. 1). They were filled with equal parts of tapwater and demiwater (pH ranging from 7.5 to 8.0). The tanks were separated from each other by cardboard partitions and placed in stands in an aircondi-

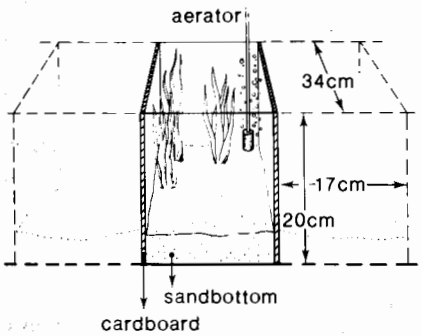

Fig. 1. Set up of tanks used to maintain and test fish.

tioned room (temperature 18-20°C). The light/dark schedule was 16L/8D (the light period started at 7:30 *a.m.*). Each row of 6 tanks was lit by a 40 Watt fluorescent lamp, mounted 15 cm above the tanks. The fish were fed twice a day on live *Tubifex* worms, *Artemia*, *Chironomus* larvae, and frozen *Artemia* or *Mysis*. Behaviour of the sticklebacks was recorded with an Esterline Angus event recorder.

2.1.2. *Behavioural tests.*

Hatching of the young in the base population occurred *ca* 8 days after fertilization. The 25 fry were isolated at first sign of aggression, *i.e.* at an average age of 34.7 ± 5.0 days after fertilization. In later generations the young were isolated on the average two weeks earlier (see section 3.2.1.).

2.1.2.1. Juvenile test.

Beginning 3 weeks after fertilization (day 42) young sticklebacks, whose sex cannot be distinguished, were submitted once a week to a "juvenile test" until sexual maturity. This test was applied earlier by SEVENSTER & GOYENS (1975), and is a variant of the male test developed by VAN IERSEL (1958). In these juvenile tests young sticklebacks were confronted with an opponent of about the same age and size, enclosed in a glass tube (2.8 cm in diameter and 20 cm high) which is wide enough for the opponent to move freely. As in the other tests, the opponents were also selected for regular mobility and absence of aggression against the experimental fish. The tube was offered alternately in the right or the left front-corner of the tank. A complete recording of the behaviour of the experimental fish was made after introduction of the opponent. If the experimental fish did not react (that is did not reach the tube) within 5 minutes after introduction, the opponent was removed and the test declared invalid. In that case the test was repeated once daily until a result was obtained for each fish, to ensure enough measurements per fish. If the experimental fish reached the tube within 5 min, its behaviour was recorded for 5 min from the time the fish had first reached the tube (valid test). Thereafter the tube was removed. After 9 weeks of testing (day 105 after fertiliztion) the tube was replaced by a wider one (4.5 cm in diameter) to allow the opponent enough freedom of movement.

2.1.2.2. Male test.

After the males had completed their first nest (judged from external appearance of the nest), their aggression was measured in a "male test". Another male with bright nuptial

colouration served as opponent in the male test. The opponent was offered in a polyacrylic plastic chamber (6 x 6 x 20 cm) that had opaque side walls and an adjustable floor to match the floor levels of the tank and the chamber. The chamber was hung outside at the front of the tank as far as possible away from the nest. The presentation of an opponent outside the tank instead of inside was based on the following considerations. First, in this way we hoped to emphasize the difference in aggression between relatively aggressive and less aggressive males; the relatively less aggressive males are expected to be even less aggressive towards an opponent outside their tank than one inside. Secondly, since the males were also submitted to "courtship tests" (see below), we made the conditions of the male test and the courtship test as different as possible in order to minimize the influence of experience on the test results. The start and duration of the male tests were defined in the same way as for juvenile tests, as described above. Tests were carried out once per week in four successive weeks. If tests were invalid the sequence was continued until four valid tests per male were obtained.

2.1.2.3. Female test.

When a juvenile female became ripe for the first time (based on external appearance of the female, *i.e.* swollen abdomen and distended cloaca) she was then subjected to "female tests". This test is almost identical to the male test, the only difference being that the opponent is a sub-adult fish of about the same size as the female in order to evoke as little sexual behaviour as possible (as in the male tests). The opponent was offered in a plastic chamber that was hung at the front of the tank, alternately left or right.

2.1.2.4. Courtship test.

A "courtship test" was interposed between two male tests, with a minimum time lag of one day between each test. A ripe female, selected for her readiness to adopt and maintain the courtship posture, served as test fish. She was offered in a glass tube (4.5 cm in diameter) as far as possible from the male's nest site, inside his tank, but not in contact with the walls in order to enable the male to circle around the female. Remaining test conditions were the same as in the other adult tests. In the literature courtship tests have been called sex tests (SEVENSTER, 1961; SEVENSTER-BOL, 1962; PEEKE, 1969), or female tests (VAN IERSEL, 1953; BAGGERMAN, 1966, 1968)!

2.1.2.5. Dominance test.

After the completion of all 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 which is unfamiliar to both, but identical to their own tank (that is, of the same size and similarly planted). Because the size of such tanks corresponds roughly to the size of a minimal territory (VAN DEN ASSEM, 1967), only one male could become dominant. The dominance test lasted until a clear dominance relation was established, that is, when one male was attacking and chasing the other (inferior) male. From then on the inferior male almost always fled when attacked, but sometimes fought back for a short time. When not attacked the inferior male stayed immobile most of the time, hidden behind a plant or floating at the surface. The dominant male, on the other hand, never fled once a clear dominance relation was established. Often he started to display nestbuilding behaviour. A clear dominance relation was almost always achieved within 10 minutes after introduction. Fighting could be of a longer duration but this occurred only with males that were still inexperienced with respect to dominance tests (see also BAKKER & SEVENSTER, 1983).

At the conclusion of a dominance test both males were returned to their own tank and stimulated with a ripe female enclosed in a glass tube in order to keep the males in reproductive condition. A male was submitted maximally to two dominance tests a day, separated by at least 4 hours.

Prior to dominance tests the males were marked by clipping the tip of the spines (2 dorsal and/or 2 pelvic spines) in various combinations. Pilot experiments had not revealed any effect of spine-clipping on the dominance status under the conditions used in this study. In these pilot experiments it appears, that when individuals taken from a group of n isolated males were tested pairwise and in all possible combinations, viz. $\frac{1}{2}n(n-1)$, the large majority of test results were in agreement with a linear order of dominance of the males concerned (see also section 3.5.1.).

The number of males in the base population was, however, too large to test the dominance relation of all possible combinations of males within the course of the study. Instead, the isolated males of the base population were divided at random into 4 groups of equal size. Within each group the males were subjected, pairwise, to dominance tests. By combining the winners as well as the losers of these first and all subsequent dominance tests, the most and the least dominant male within each group was eventually identified. Finally, the most and the least dominant males of each of the 4 groups were tested against each other in the 28 possible pairwise combinations in order to assess a dominance order of these 8 males.

For the sake of convenience a summary of the different test designs is presented in Fig. 2. To avoid superfluous use of terms I will call the chamber (used to enclose the opponent in the female and male tests) also tube from now on.

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

Fig. 2. Outline of behavioural tests performed in the study.

2.1.3. *Measures of aggression.*

To quantify aggressive behaviour one can make use of latencies, frequencies or durations of aggressive elements. The use of a particular measure is often imposed by the circumstances. By definition biting is the most extreme manifestation of aggressiveness. The way in which biting was quantified in this study was imposed by the attacking behaviour of juvenile sticklebacks during the juvenile tests. The aggressiveness of the juveniles manifested itself in bouts of variable lengths, during which the experimental fish was biting (snapping jaws or open mouth) or bumping (closed mouth) nearly uninterrupted against the tube and apparently directed at the opponent. Part of these biting-bumping bouts ended with a more or less intense bite, after which contact with the tube was broken off. Because biting and bumping could only be distinguished with great difficulty, the total duration of the biting-bumping bouts was taken as a measure of aggressiveness. Because juveniles and adult females were relatively susceptible to

disturbances (caused *e.g.* by movements of the observer, or sudden movements of the opponent) and then swam away from the tube, the duration of biting-bumping was related to the period they stayed within a fish-length distance of the tube (tube-period). This measure I will call "% biting-bumping time in the tube-period".

This correction was not necessary in the case of adult males. On the contrary, it would rather increase the relative duration of biting-bumping disproportionately, especially when there is some courting, since the male is then often away from the tube (of course this happens mainly during courtship tests, but may also happen during male tests). Aggressiveness in the male and courtship tests will therefore be expressed as "% biting-bumping time in the test-period". In the adult male tests the 5 min test-period started after the first arrival at the tube or after the first zigzag bout of the male, whichever came first. Otherwise, a number of tests would be classified as invalid because courting males not always cross the critical distance from the tube within 5 minutes.

The % biting-bumping time in the tube-period is not an isolated quantity. It is significantly correlated with other measures of aggression, as is shown in Table 1 for juvenile males of the base population. In fact, all the presented measures of aggression are significantly correlated with each other to various degrees, indicating that the behavioural measures may be related in a causal sense (cf. WOOTTON, 1971). In section 4.5. I will return to this topic.

TABLE 1. Correlation matrix for some measures of aggression of juvenile males of the base population (n = 42), based on individual means of all juvenile tests

	1	2	3	4	5	6
1. % Biting-bumping time in tube-period	1	−0.53	−0.64	+0.54	+0.75	+0.83
2. Meet latency		1	+0.49	−0.34*	−0.39	−0.43
3. Attack latency			1	−0.78	−0.69	−0.47
4. % At tube in test-period				1	+0.55	+0.35*
5. Mean bout length of biting-bumping time					1	+0.59
6. Number of intense bites						1

Meet latency = time between introduction of the tube and first arrival at the tube (the maximal time possible is 300 sec). Attack latency = time between first arrival at the tube and first attack (the maximal time possible is 300 sec). The other measures are explained in the text. All correlation coefficients differ from 0 at the 1% level, except * = $p < 0.05$ (t-test, 2-tailed).

A criticism often made of behavioural tests such as the ones used here, is that such tests poorly represent natural situations. Some importance has to be attached to this criticism in the case of the juvenile tests, because juvenile sticklebacks usually are moving in schools. However, in the present study the aggression of juveniles in groups of fixed size was also assessed by recording the total number of bites and bumps observed in groups of young sticklebacks. It will be shown that there exists close agreement between the level

of aggression in these groups and the mean level of aggression of their isolated full sibs, as measured in juvenile tests (see section 4.6.1.).

2.1.4. *Methods of quantitative genetics.*

Using the methods of quantitative genetics the total or phenotypic variance (V_P) can be split up into components attributable to different causes. The first step requires division of the phenotypic variance into two components, the genetic variance (V_G) and the environmental variance (V_E); $V_P = V_G + V_E$ (see FALCONER, 1981 for a more extensive treatment, and for a justification of the simplifications involved). In the population under study the relative importance of the different genotypes for determining differences in phenotypes is given by the ratio of genotypic to phenotypic variance, V_G/V_P (called heritability in the broad sense and often denoted by the false and thus confusing description: "degree of genetic determination of a character").

An appropriate way to determine the V_E component is by means of inbred lines, or their crosses. In the case of sticklebacks these are not available. However, when repeated measurements are made with the same individuals, a certain component of V_E can be estimated. By an analysis of variance the component of the variance due to temporary environmental circumstances (V_{Es} = special environmental variance) can be calculated. The component of V_E due to permanent environmental circumstances (V_{Eg} = general environmental variance), however, cannot be separated from permanent differences between individuals that are due to genetic causes, V_G.

The intra-group correlation between repeated measurements of the same individuals is known as the repeatability (r) of the character: $r = (V_G + V_{Eg}) / V_P$. When V_{Eg} is zero the repeatability is equal to the heritability (in the broad sense), and so the former sets an upper limit to the value of the latter. Circumstances between experiments were held as identical as possible, to minimize V_{Eg}. If repeated measurements are averaged, then the phenotypic variance will be: $V_{P(n)} = V_G + V_{Eg} + 1/n\ V_{Es}$. By increasing the number of measurements per individual the phenotypic variance is reduced and thus the heritability value will increase.

The genetic variance can also be split up into components. These components are: the additivic variance (V_A), the dominance variance (V_D) and the interaction variance (V_I). If we ignore the interaction component, which usually is the smallest, and make some further assumptions (see FALCONER, 1981), the genetic variance can be expressed as: $V_G = V_A + V_D$. The fixable part of the genotypic variance (that is, the part that is susceptible for directional selection), V_A, is of special interest here, because the ratio V_A/V_P (h^2, heritability in the narrow sense) provides us with a predictor of the rate of response to directional selection for a character (see section 3.2.4.).

In the base population of this study heritability estimates can also be made from the resemblance between full sibs. The correlation of full sibs can be calculated with an analysis of variance, and expressed, like the repeatability, in an intra-class correlation coefficient (t_{FS}). It can be shown that the full sib correlation (t_{FS}) is equal to $(\frac{1}{2}V_A + \frac{1}{4}V_D) / V_P$ (see FALCONER, 1981). Consequently $2t_{FS}$ gives too high an estimate of the heritability in the narrow sense; it merely sets an upper limit to h^2.

2.2. Juvenile aggression.

The aggression of juvenile fish, as measured with the juvenile test, I will call "juvenile aggression". Males and females do not differ in aggressiveness during their juvenile stage (Table 2). It should be realized

TABLE 2. Juvenile aggression (expressed as the % biting-bumping time in the tube-period) as a function of test-age (in weeks after fertilization) of juvenile males (n = 43) and juvenile females (n = 57) of the base population

Age	Mean juvenile aggression ± S.D. juvenile ♂♂	juvenile ♀♀
6	23.3 ± 12.6 (5)	30.2 ± 22.1 (12)
7	26.8 ± 18.9 (29)	31.3 ± 20.4 (35)
8	27.0 ± 22.4 (27)	35.1 ± 21.8 (36)
9	33.1 ± 18.7 (27)	29.2 ± 19.3 (35)
10	20.9 ± 19.3 (17)	35.1 ± 24.1 (27)
11	34.3 ± 19.1 (14)	27.9 ± 25.2 (22)
12	21.2 ± 18.1 (28)	27.3 ± 21.2 (40)
13	24.4 ± 17.1 (24)	24.8 ± 16.6 (38)
14	20.2 ± 20.9 (14)	20.0 ± 13.4 (28)
15	20.2 ± 19.4 (15)	22.9 ± 19.9 (22)
16	21.6 ± 15.8 (23)	25.6 ± 19.2 (38)
17	16.5 ± 14.7 (20)	22.0 ± 17.0 (39)
18	20.0 ± 17.8 (18)	15.9 ± 17.1 (29)
19	9.9 ± 14.0 (7)	14.8 ± 11.8 (16)
20	10.5 ± 15.2 (4)	26.4 ± 21.9 (6)
21	6.2 ± 4.9 (8)	16.0 ± 13.0 (13)
22	13.5 ± 13.2 (6)	12.9 ± 14.4 (14)
23	14.1 ± 10.9 (5)	11.1 ± 6.4 (10)
24	9.6 ± 10.3 (6)	17.5 ± 15.4 (9)

Numbers of fish on which means are based in parentheses.

that the values at the tail-end of these age ranges are less reliable, since by this time some individuals have already matured. The variable number of measurements per age-week was primarily due to this variation in maturity-age (Fig. 3). By day 233 (after fertilization) 88.9% of the juveniles had become sexually mature. The mean date of maturation

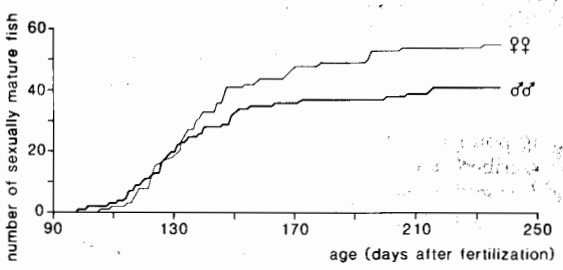

Fig. 3. Variation in the age at which individuals in the base population reach sexual maturity.

(until day 233) was the same for both males and females (138.3 ± 28.5 and 140.0 ± 26.8 days, respectively; Mann-Whitney U test, 2-tailed, $p > 0.25$).

The above presented findings show that three-spined sticklebacks can readily be brought to sexual maturity when reared at long daylengths (16L/8D) and high temperatures (18-20°C). This holds not only for offspring of the freshwater population used in the present study but also for those of anadromous populations (BAKKER & FEUTH-DE BRUIJN, unpublished results). These results seem to contradict those of BAGGERMAN (1957), which suggest an inhibition of maturation of long daylengths combined with high temperatures in young shorter than 2.2 cm. In order to obtain sexually mature fish most researchers exposed therefore their laboratory-bred young temporarily to short daylengths and low temperatures (*e.g.* MCPHAIL, 1977; HAGEN & MOODIE, 1979; HAGEN & BLOUW, 1983). In the light of the results of the present study this seems a superfluous step.

Secondly, the actual number of tests per age-week varied because of a variation in the responsiveness of the juveniles to the juvenile test (Fig. 4). Juvenile males arrived on the average within 5 min at the tube in $51.6\% \pm 34.9$ of the tests (mean responsiveness per juvenile male $\pm$ S.D.), while the mean responsiveness of juvenile females per female was somewhat greater, viz. 63.8 ± 33.5 (a difference significant at the 5% level, Mann-Whitney U test), due to a higher fraction of juvenile females that always reacted within 5 min, as shown in Fig. 4.

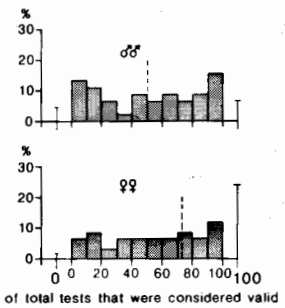

Fig. 4. Frequency distributions of the mean responsiveness of juvenile males (n = 45) and juvenile females (n = 58) of the base population during juvenile tests. Fish with a mean responsiveness of 0% (not any valid test) or 100% (valid tests only) are classified separately. ┆ = median.

A most intriguing point is the obvious decline of juvenile aggression with age. This decline cannot be ascribed to habituation to the test situation, since diminishing of the test frequency in later generations did not alter this decline (see section 3.3.3.).

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. In view of the variation in the age of sexual maturity and the decline of juvenile aggression with age, an appropriate juvenile aggression score might be the mean deviation of the individual course of juvenile aggression with age, relative to the mean course of all juveniles. A more convenient score used further on in the present study is the mean juvenile aggression during the tests prior to week 15 (that is, until day 98 after fertilization, the minimum age of sexual maturity). In the base population at least this *juvenile aggression score* (JAS) is indeed a reliable measure of juvenile aggression, as supported by the high correlation ($r = 0.93$, for both juvenile males and juveniles females) between JAS and the mean deviation mentioned above.

Again there is no difference between males and females, as reflected by the similar frequency distributions of their juvenile aggression scores (Fig. 5). The mean score of juvenile males was 24.2 ± 14.8 ($n = 40$) and of juvenile females 27.5 ± 17.9 ($n = 55$), a nonsignificant difference (t test, 2-tailed, $p > 0.20$).

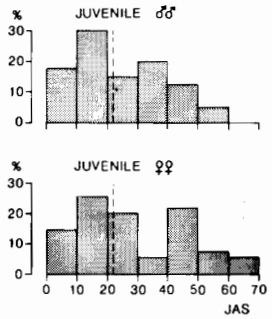

Fig. 5. Frequency distributions of the juvenile aggression scores (JAS) for juvenile males ($n = 40$) and juvenile females ($n = 55$) of the base population. | = median.

The finding that the level of aggression in males and females does not differ during their juvenile stages, is in accordance with the results of experiments of WAI & HOAR (1963), who did not find a difference in aggression between gonadectomized males and females under long day photoperiods. More directly comparable to the present study is a study by SEVENSTER & GOYENS (1975). They also failed to find a difference in aggression (measured in a test identical to the juvenile test) between juvenile males and females at an average age of 108 days after hatching. Another study is that of HUNTINGFORD (1979), in which it was concluded, on the basis of a factor analysis, that the responses of male and female three-spined sticklebacks to a strange conspecific just prior to the sexual phase were roughly similar. However, aggression of nearly mature males was in this case higher

than that of nearly mature females. This may be due to the different testing methods used (*e.g.* she presented adult males lacking breeding colours as opponents) and/or the advanced age of the subjects at testing.

2.3. Aggression of adult females.

The aggressiveness of adult females (the adult stage starting by definition with the readiness to spawn for the first time) was measured with the female tests and termed "female aggression". The course of mean female aggression after maturation as a function of age at testing is shown in Table 3. At later ages female aggressiveness appears to decline: week 24 *vs* week 25, Wilcoxon matched-pairs signed-ranks test, 2-tailed,

TABLE 3. Female aggression after maturation (expressed as the % biting-bumping time in the tube-period) as a function of test-age (in weeks after fertilization) of females (n = 46) of the base population

Age	Mean female aggression ± S.D.
21	16.5 ± 11.9 (16)
22	14.9 ± 12.2 (33)
23	15.9 ± 13.0 (33)
24	16.4 ± 13.9 (34)
25	10.9 ± 9.7 (24)
26	8.2 ± 12.6 (12)

Numbers of fish on which means are based in parentheses.

$n = 20$, $0.02 < p < 0.05$. This decline is not understood; there is no systematic decline of mean female aggression as a function of time elapsed since the onset of sexual maturity (Table 4), nor is there a decline in mean aggression with increasing age at which maturity starts (Spearman rank correlation coefficient = -0.04, n = 46).

At the conclusion of the female tests the degree of ripeness was judged, based on external appearance of the females, and expressed roughly on a threepoint scale:
ripe: female clearly ready to spawn soon (*i.e.* swollen abdomen and distended cloaca);
non ripe: female with flat or sunken belly, and
half ripe: female somewhere between ripe and non ripe conditions.
In Table 5 female tests are arranged with respect to the degree of ripeness of the females. Ripe females tend to be less aggressive than nonripe or half ripe females. This tendency is even more pronounced when we split the tests of ripe females according to whether or not they showed the head-up courtship posture during the test. In the female tests the head-up posture was only observed in ripe females. But WOOTTON (1974a, b) claims that in the presence of a courting male in some females the head-up posture can be elicited throughout the interspawning interval, although latencies to head-up posture are lowest just before and after spawning.

TABLE 4. Female aggression (expressed as the % biting-bumping time in the tube-period) as a function of time elapsed since the onset of sexual maturity (*i.e.* readiness to spawn for the first time) of females (n = 46) of the base population

Week after sexual maturity	Mean female aggression ± S.D.
1	16.2 ± 15.0 (13)
2	15.5 ± 10.8 (17)
3	19.1 ± 14.0 (22)
4	11.8 ± 12.1 (25)
5	12.5 ± 10.7 (24)
6	17.2 ± 14.8 (21)
7	13.3 ± 11.7 (16)
8	13.6 ± 13.4 (10)

Numbers of fish on which means are based in parentheses.

TABLE 5. Female aggression (expressed as the % biting-bumping time in the tube-period) as a function of the degree of ripeness of females (n = 46) of the base population

Degree of ripeness	Mean female aggression ± S.D.
ripe	11.7 ± 12.2 (37)
	+head-up: 10.5 ± 13.6 (15)
	-head-up: 12.6 ± 11.3 (22)
half ripe	15.9 ± 12.9 (39)
non-ripe	15.2 ± 12.8 (81)

The tests on ripe females are split according to whether or not the head-up posture is shown during the test. For definitions see text. Numbers of tests on which means are based in parentheses.

The reduction of aggression in ripe females is, however, not significant: scores for the same females when ripe or nonripe are 10.0 ± 10.6 and 13.9 ± 10.2, respectively; Wilcoxon matched-pairs signed-ranks test, n = 22, $p > 0.05$. Anyhow, because the proportion of ripe females during the female tests wanes slightly with age, this reduction of aggression in ripe females counteracts the reduction of aggression with age mentioned above.

The outcomes of the various female tests for each female are, as with the juvenile tests, averaged to get an individual *female aggression score* (FAS). The frequency distribution of these scores is shown in Fig. 6. The low aggression scores are relatively frequent with a mean FAS of 14.5 ± 11.1 (n = 46).

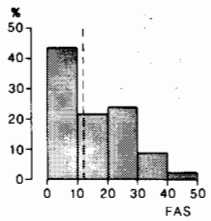

Fig. 6. Frequency distribution of the female aggression scores (FAS) for females (n = 46) of the base population. | = median.

Finally, the mean responsiveness of the females per female in the female tests averaged 81.9% ± 32.1. Of the 49 females tested 32 (65.3%) always reacted within 5 min after presentation of an opponent.

2.4. Aggression of adult males.

The aggression of adult males (the adult stage starting by definition with the completion of the first nest) was measured in three different situations, viz. against a rival at the front-pane of his own tank (male test), against a ripe female in his own tank (courtship test), and against a rival in a tank unfamiliar to both males (dominance test). The next three sections focus on the variation of aggression in each of these three different situations and then the levels of aggression in the different situations are compared in section 2.5.1.

2.4.1. *Territorial aggression.*

I term the aggression of males measured in the male tests "territorial aggression". This remains fairly constant with the age of the males (Table 6), and also after the time elapsed since they finished their first nest

TABLE 6. Territorial aggression after first nestbuilding (expressed as the % biting-bumping time in the test-period) as a function of test-age (in weeks after fertilization) of males (n = 36) of the base population

Age	Mean territorial aggression ± S.D.
21	42.9 ± 20.2 (11)
22	37.3 ± 21.7 (27)
23	36.9 ± 21.8 (32)
24	36.8 ± 21.4 (31)
25	38.8 ± 19.9 (25)
26	34.6 ± 28.4 (9)

Numbers of males on which means are based in parentheses.

(Table 7), which is in concordance with literature (*e.g.* BAGGERMAN, 1957; PEEKE *et al.*, 1969). Under these test conditions territorial aggression appears to be slightly higher later on in the sexual phase (Table 7).

TABLE 7. Territorial aggression (expressed as the % biting-bumping time in the test-period) as a function of time elapsed since the onset of sexual maturity (*i.e.* completion of the first nest) of males (n = 36) of the base population

Week after first nestbuilding	Mean territorial aggression ± S.D.
1	31.8 ± 30.2 (6)
2	37.2 ± 20.2 (11)
3	33.7 ± 22.3 (16)
4	34.4 ± 19.6 (22)
5	39.0 ± 18.9 (22)
6	34.5 ± 19.2 (18)
7	44.5 ± 25.8 (18)
8	40.9 ± 23.6 (11)
9	39.5 ± 16.5 (8)

Numbers of males on which means are based in parentheses.

The outcomes of the male tests were averaged for each male to yield individual *territorial aggression scores* (TAS). As with the other aggression scores, the territorial aggression scores are highly variable in the base population (Fig. 7; mean = 38.1% ± 18.3, n = 36).

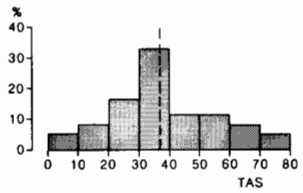

Fig. 7. Frequency distribution of the territorial aggression scores (TAS) for males (n = 36) of the base population. | = median.

As with female aggression scores, no correlation was found between TAS and age of sexual maturity (r_S = -0.13, n = 36, not significant).

Although the behavioural tests were standardized as much as possible, certain environmental variables such as the distance between the nest and the opponent cannot be controlled without interventions of the observer. In the small tanks used in this study the

maximum possible distance between the nest and the opponent is about 30 cm. Since for distances shorter than 30-40 cm the aggressiveness of the territory owner decreases with increasing distance from the nest (VAN IERSEL, 1958, a result confirmed by SYMONS, 1965; BLACK 1971; HUNTINGFORD, 1976b; ROWLAND, 1983a, b), it should be expected that the variation in location of the nest contributes to the variation in territorial aggressiveness. Of the 36 males of the base population 24 did not shift their nest during the testing period. Of these males 10 situated their nests in the front half of the tanks and thus, the opponent had to be presented closer to the nest than in the case of the 14 males that had their nests in the back half of their tanks. The mean territorial aggression scores of these two groups were 46.5% ± 16.9 and 35.8% ± 17.1, respectively. This difference, though not significant (Mann-Whitney U test), is in accordance with the literature.

The responsiveness of the males in the male test is high: 32 out of the 37 males (86.5%) always reacted within 5 min after the presentation of the opponent. The mean responsiveness per male averaged 93.9% ± 19.5.

2.4.2. *Courtship aggression.*

The aggression tests (juvenile, female and male tests) are not the only situations in which aggression can be elicited. Also during courtship tests aggressive behaviour of the male can make up a considerable part of the test time. This aggressive behaviour directed against a ripe female I will call "courtship aggression".

In the base population courtship aggression showed a slight decline with test-age (Table 8), but when courtship aggression is presented as a function of time elapsed since the onset of sexual maturity the decline is much more pronounced (Table 9). These results have to be interpreted carefully. For technical reasons courtship tests could not always be carried out as soon as the males became mature. Some males were tested later than others and in Table 10, therefore, the first courtship test rather than the onset of sexual maturity is taken as the point of reference. The decline in aggression with increasing test number is especially evident if the first and the second courtship tests are compared (Wilcoxon matched-pairs signed-ranks test, $p<0.01$), although the overall trend is also significant (Friedman two-way analysis of variance, $df=2$, $p<0.001$). For the interested reader a possible explanation for this decline is analysed below.

Before the first courtship test each male had been subjected to several aggression tests: a series of juvenile tests and at least one male test. In these tests the opponent was offered in front of the male's tank. In the courtship tests the glass tube with a ripe female was offered as far as possible from the male's nest. Thus, if the nest was situated in the back-half of the tank the female was offered in the front-half, and so, in terms of the position of the opponent, these courtship tests more resembled the aggression tests than courtship tests in which the nest was situated in the front-half. It is conceivable that in these cases

TABLE 8. Courtship aggression after first nestbuilding (expressed as the % biting-bumping time in the test-period) as a function of test-age (in weeks after fertilization) of males (n = 37) of the base population

Age	Mean courtship aggression ± S.D.
22	25.6 ± 30.2 (18)
23	24.9 ± 27.4 (33)
24	13.6 ± 18.8 (33)
25	9.2 ± 13.6 (33)
26	18.1 ± 22.3 (18)

Numbers of males on which means are based in parentheses.

TABLE 9. Courtship aggression (expressed as the % biting-bumping time in the test-period) as a function of time elapsed since the onset of sexual maturity (*i.e.* completion of the first nest) of males (n = 37) of the base population

Week after first nestbuilding	Mean courtship aggression ± S.D.
1	39.0 ± 16.8 (6)
2	20.1 ± 24.3 (10)
3	24.3 ± 23.3 (14)
4	21.9 ± 29.0 (23)
5	15.7 ± 23.4 (22)
6	10.7 ± 15.9 (21)
7	17.3 ± 23.8 (19)
8	9.6 ± 17.4 (10)
9	6.5 ± 11.6 (6)

Numbers of males on which means are based in parentheses.

TABLE 10. Courtship aggression after first nestbuilding (expressed as the % biting-bumping time in the test-period) as a function of test number of males (n = 37) of the base population

Test number	Mean courtship aggression ± S.D.
1	30.3 ± 29.3 (37)
2	16.2 ± 22.6 (35)
3	11.6 ± 15.5 (35)
4	10.9 ± 16.5 (33)

Numbers of males on which means are based in parentheses.

the males displayed more aggressive behaviour in their first courtship test because position might have become associated with a male intruder. Of the 37 males of the base population 31 can be used to analyse this point (6 changed nest position between the first and second courtship test). Males nesting in the back-half (n = 17) spent on the average 36.3% of the test-time biting and bumping the female during their first courtship test and 16.4% during the second test (Wilcoxon matched-pairs signed-ranks test, 2-tailed, $p<0.01$). In the 14 males nesting in the front-half these percentages were 24.1% and 14.3%, respectively (Wilcoxon matched-pairs signed-ranks test, 2-tailed, $p = 0.02$). The more pronounced difference between the first and second test in males whose nests were situated in the back supports the locality association hypothesis mentioned above of these males in the first test. The difference between the first test of males nesting in the front and those nesting in the back is, however, insignificant (Mann-Whitney U test, $p>0.05$).

In conclusion, the locality association hypothesis may partly explain the decline in courtship aggression with increasing test number, but other, unknown causes must be involved in this decline as well.

As in the other behavioural tests, *courtship aggression scores* (CAS) were obtained by calculating an average for each male during the various courtship tests and these are highly variable in the base population with low scores being most frequent (Fig. 8; mean CAS = 18.3% ± 19.1, n = 37).

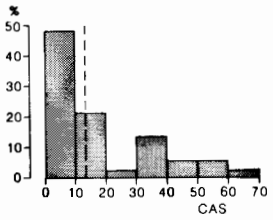

Fig. 8. Frequency distribution of the courtship aggression scores (CAS) for males (n = 37) of the base population. | = median.

The responsiveness of reproductive males in courtship tests is high: 35 out of the 37 males (94.6%) always reacted within 5 min after the introduction of a ripe female. The mean responsiveness per male averaged 97.3% ± 13.5.

2.4.3. *Dominance.*

As explained in section 2.1.2.5. the most dominant and the least dominant (or most inferior) male were identified in each of the four groups, in which the males of the base population were divided. These 8 males were tested pairwise in tanks that were unfamiliar to both contestants. Following the results of the seven contests each male had to undergo, the eight males were arranged in a linear order of dominance, *i.e.* in such a

way that the least number of test outcomes deviating from linearity was obtained. The outcome of all but one of the 28 tests fitted in with a linear order of dominance. The one "wrong" test outcome may be ascribed to an experience of losing, since the "wrong" loser lost against the most dominant male in the test before (see also BAKKER & SEVENSTER, 1983). The linear order of dominance in the present study is different from the classical hierarchy-concept like that of SCHJELDERUP-EBBE (1922) (see BAKKER & SEVENSTER, 1983 for a more extensive treatment), because individual recognition was ruled out; each male was confronted only once with the same opponent. Yet experience plays a role (see BAKKER & SEVENSTER, 1983), since the loser of a contest is likely to loose in the next contest, other things being equal. This effect of experience in itself could determine an order of dominance. However, it can be shown that the order is not determined only by the sequence of the dominance tests. Therefore, the rank of a male in the linear order of dominance reflects, in a quantitative way, his inherent ability to dominate others (the higher his rank, the greater his dominance ability). This will be called his *dominance ability* or *dominance* from now on.

With a view to the genetic selections, dominance tests were carried out on adult females. Altogether 48 females of the base population, who had been in isolation so far, were subjected pairwise to a dominance test, like the males. Eight out of the 24 tests gave clear-cut dominance relationships within a pair of females, comparable to the relation within pairs of males. The inferior females darken considerably, and in this respect resemble the males whose inferior-status is also accompanied by a temporary fading and darkening of their colouration. The dominant fish (males and females) show the reverse, though this is less striking. Such clear-cut dominance relations have not been described so far for females of the three-spined stickleback but are reminiscent of the territoriality of adult females of the nine-spined stickleback (*Pungitius pungitius* (L.)) in captivity, as described by MORRIS (1958), and adult females of the three-spined stickleback (LI & OWINGS, 1978a; SEVENSTER, pers. comm.).

In most cases dominance relations between females were not so clear-cut. In 16 of the 24 tests no clear dominance relation was established (at least not within three quarters of an hour after introduction), although in 12 of 16 tests one of the pair initiated more attacks than the other and possessed a lighter colour. In the 4 remaining tests aggression was either absent or both females bit each other equally often.

The low level of fighting is also reflected in the incidence of round-about fighting in dominance tests with females. This form of fighting is

seen between well-matched males in the laboratory and in the wild, in situations where neither male has priority (see BAKKER & SEVENSTER, 1983). In our dominance tests roundabout fighting was not confined to fighting males, as WOOTTON (1976) stated, but occurs in females as well, though at a low level (viz. in 5 out of 24 dominance tests; 21%).

Though dominance in adult females is itself an interesting topic of study, its use as a criterion for selection seemed doubtful, since in most pairs no clear-cut dominance relation was established within a short period of time. Therefore we abandoned further dominance tests with females.

2.5. Comparisons between test paradigms.

After investigating variation in aggressiveness of three-spined sticklebacks in different situations (sections 2.2., 2.3. and 2.4.), this section (2.5.) focuses on the interrelations between the aggression levels exhibited in these different test situations.

2.5.1. *Aggression tests.*

To facilitate comparison of aggression exhibited in juvenile, male and female tests (aggression tests) as a function of age the data of Tables 2, 3 and 6 have been plotted in Fig. 9. This shows that the level of aggression of males and females does not differ during the juvenile stage, as

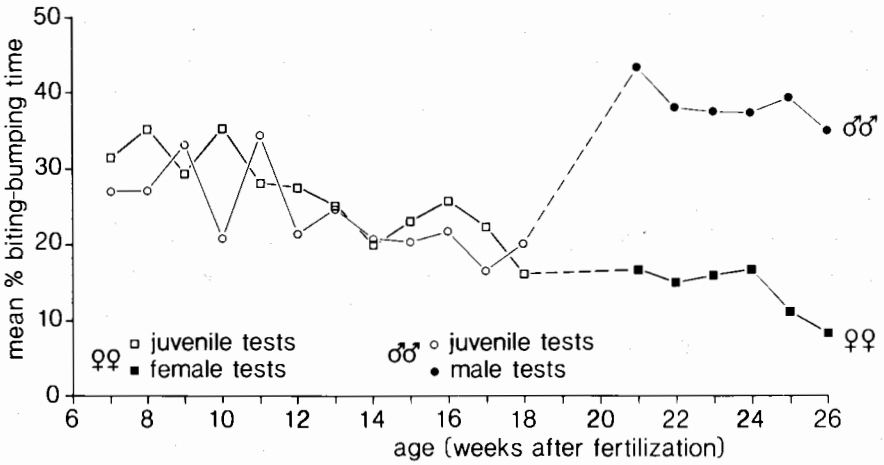

Fig. 9. Mean level of aggression as a function of age in the base population. Aggression expressed as % biting-bumping time in the tube-period, except for adult males where it is related to the total test time.

already noted in section 2.2. Sexual maturity of the females is not accompanied by a clear change in aggressiveness. The slight but persistent decline of aggression with age observed during the juvenile stage continues into the adult stage. If both stages are compared, like SEVENSTER & GOYENS (1975) did, a difference between the levels of aggression is found. This is a long-term effect, for if the last juvenile tests are compared with the first female tests of the same individuals, there is no significant difference between the frequency distributions of the levels of aggression in those tests (Fig. 10; Wilcoxon matched-pairs signed-ranks test. n = 30, 2-tailed, $p > 0.40$). The gradual decline of aggression with age is not understood.

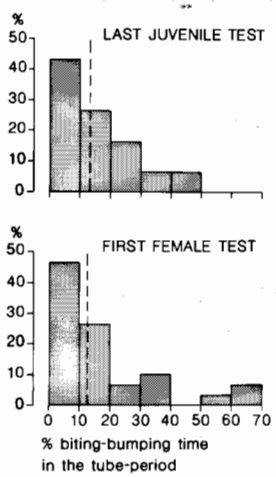

Fig. 10. Frequency distributions of the levels of aggression during the last juvenile test and the first female test for 30 females of the base population with an age difference ≤ 4 weeks between these two tests. | = median.

In males sexual maturity is accompanied by an increase in aggressiveness (Fig. 9), which is in accordance with the literature (for a review see below). Though it is tempting to discuss here the hormonal influences on aggressive behaviour in both developmental stages, I will postpone this topic to a later section (section 4.7.).

In contrast with literature concerning aggressiveness of adult females, much attention has been paid to the aggressiveness of adult stickleback males. Castration was used by CRAIG-BENNETT (1931) and IKEDA (1933) to study the effects of gonadal hormones on the secondary sexual characters and reproductive behaviour of stickleback males. This was continued by HOAR (1962a, b) who studied the change of aggressiveness coinciding with sexual maturity. HOAR subjected male three-spined sticklebacks to castration and hor-

mone treatment prior to and during their reproductive cycle. BAGGERMAN (1966) was the first to study systematically aggressiveness in intact male sticklebacks before, during and after the sexual phase. Further study of the change of aggressiveness coinciding with sexual maturity includes the work of BAGGERMAN (1968), WOOTTON (1970), SEVENSTER & GOYENS (1975) and HUNTINGFORD (1976a). Though all of these workers measured aggressiveness differently, they all found an increase in aggressiveness of males at sexual maturity. Changes in aggressiveness therefore seem to be rather consistent and independent of testing methods. Such independency of methods was also found of changes in male aggressivenes during the parental phase (WOOTTON, 1971).

In most literature on changes of male aggressiveness at sexual maturity territorial aggressiveness is compared with aggression during the "pre-breeding" or "pre-nest phase", *i.e.* the period prior to nest-building, when adult, non-reproductive males in winter conditions (stored at 8L/16D and 10°C) are exposed to spring conditions (16L/8D and 20°C) (see *e.g.* BAGGERMAN, 1957, 1972 for more detailed information on this subject). In studying pre-breeding aggression often males in reproductive condition are used as opponents. Only SEVENSTER & GOYENS (1975) compared aggression during the juvenile and sexual phases, as in the present study. Moreover, only they used juvenile fish as opponents to study juvenile aggressiveness. On the other hand, the level of aggression before the sexual phase appears to be little affected by the kind of opponent used (see WOOTTON, 1970).

In this section the mean levels of aggression of fish from the base population in the juvenile, female and male tests were compared. In the next section I investigate the extent to which aggression scores of individuals obtained in different test situations are correlated with each other.

2.5.2. *Phenotypic correlations.*

More information about the interrelations of aggressiveness in the different test situations is provided by comparison of individual aggression scores between situations. These are expressed as Spearman rank correlation coefficients since normal distributions cannot be assumed for all scores. If for a certain fish less than 4 valid juvenile tests and/or less than 3 valid adult tests were available, the data were considered insufficient and were omitted from the correlation analysis.

The similarity in level and distribution of aggression of juvenile and adult females is demonstrated once more by the rather high correlation between their aggression scores (Table 11). The correlations between the aggression scores of males (viz. JAS, TAS, CAS and dominance) are much lower. Only territorial aggression and courtship aggression are significantly correlated, as already found by SEVENSTER (1961).

Further, HUNTINGFORD (1976b) compared aggression of sticklebacks during the sexual and parental phases using a method comparable to the male tests in the present study, and found that the levels of aggression in these two test situations were positively correlated (r_S ranging from 0.58 to 0.66, all significant).

TABLE 11. Spearman rank correlation coefficients between aggression scores obtained with different behavioural tests in the base population

	JAS	TAS	CAS
FAS	+0.61 (33)**	—	—
TAS	+0.24 (23)^{ns}	1	—
CAS	+0.14 (23)^{ns}	+0.30 (33)*	1
D	-0.14 (7)^{ns}	+0.25 (7)^{ns}	+0.45 (7)^{ns}

JAS = juvenile aggression score, FAS = female aggression score, TAS = territorial aggression score. CAS = courtship aggression score, D = dominance. Numbers of fish in parentheses. ns = $p>0.05$, * = $p<0.05$, ** = $p<0.01$.

One must realize that these correlations represent phenotypic correlations. The phenotypic correlation can be split up, like the phenotypic variance, into components attributable to different causes, viz. the environmental correlation and the genetic correlation. Ultimately we are interested most in the extent to which such correlation is genetically based. The genetic correlation cannot be determined from the phenotypic correlation alone. Even its sign can be different from that of the phenotypic correlation (see FALCONER, 1981 for some examples). The topic of genetic correlation will be treated in more detail in chapter 4.

2.6. Estimates of heritabilities.

In this section (2.6.) we return to the variation of aggressiveness in each of the different test situations (see section 2.2., 2.3. and 2.4.), but viewed from a different angle. Hitherto we were occupied with the influence of nongenetic variables, like age, degree of ripeness, distance from the nest, *etc.*, on the variation of aggressiveness in each test situation. Here I try to relate the variation of aggression to genetic variation of the fishes. For that purpose the test results of the base population can be looked upon as measurements of individuals with various degrees of relatedness:

1. repeated measurements of aggressiveness made on the same (= genetically identical) individuals (section 2.6.1. repeatability);
2. aggression scores of offspring from the same parental pair (section 2.6.2. full sib correlation).

In these ways estimates can be made of the heritability of aggressiveness in the different test situations, estimates which indicate the feasibility of genetic selection for aggressiveness exhibited in a certain test.

2.6.1. *Repeatability.*

When tests are repeated, one rarely expects the same result. Among other things, this is caused by the variability of environmental influences that cannot be kept constant. The variance due to such temporary fluctuations can be separated from the total phenotypic variance by an analysis of variance. What is left is the variance due to permanent genetic and environmental differences between individuals. When this latter value is divided by the total phenotypic variance one obtains the measure of "repeatability". As for the phenotypic correlations, not all individuals of the base population have been used in the present analysis. For juvenile aggression only fish with 6, 7 or 8 valid juvenile tests were selected and only one juvenile fish of each parental pair was chosen randomly in order to obtain a genetically heterogeneous sample comparable to those used in other calculations. Likewise, repeatabilities of adult aggressiveness shown during the various tests are calculated with genetically non-related individuals with four valid tests each (male, female or courtship tests). Because of the general decline of juvenile aggression with age, calculations are based on deviations from the mean at each age (see Table 2). The analysis of variance, especially with samples of unequal size (*i.e.* fish with unequal numbers of tests), is treated *e.g.* by SNEDECOR (1956).

The results of the analyses of variance are summarized in Table 12. Measurements of aggressiveness by the various behavioural tests, except

TABLE 12. Repeatabilities for the levels of aggression during the different behavioural tests in the base population

	n_f	n_t	Repeatability	F	p
Juvenile aggression ♂♂	18	6.72	0.37	4.99	<0.01
Juvenile aggression ♀♀	20	6.70	0.65	13.35	<0.01
Female aggression	19	4.00	0.77	14.11	<0.01
Territorial aggression	19	4.00	0.62	7.92	<0.01
Courtship aggression	20	4.00	0.54	5.70	<0.01

For explanation see text. n_f = number of fish, n_t = number of tests (weighted average), F = variance ratio, p = probability.

for juvenile tests on males, show a rather high degree of repeatability, that is, the influence of differences in environmental circumstances between tests is limited.

The comparatively low repeatability for juvenile aggression in males appears to be caused by the transition from week 8 to week 9 (after fertilization) as Table 13 clearly illustrates, since all other transitions have higher repeatabilities. Why this is so, remains obscure.

TABLE 13. Repeatabilities for the levels of juvenile aggression in various, successive test weeks of juvenile males of the base population

Test weeks	n_f	n_t	Repeatability	F	p
7 to 15	18	6.72	0.37	4.99	<0.01
7 to 10	18	3.10	0.36	2.73	<0.01
7 and 8	13	2.22	0.70	6.19	<0.01
8 and 9	14	2.14	0.37	2.23	ns
9 and 10	8	2.36	0.71	6.81	<0.01
10 to 15	18	3.43	0.65	7.46	<0.01
15 to 19	16	3.23	0.54	4.77	<0.01

For symbols see previous table.

Among the adult tests, the repeatability for courtship aggression is lowest. This was expected since courtship aggressiveness remained less constant after sexual maturity than territorial and female aggressiveness (cf. Tables 4, 7 and 9). Further, the behaviour of the ripe female during courtship tests might have lowered the repeatability for courtship aggression. If carefully selected, a ripe female provides an external situation of great constancy, independent of the male's behaviour (SEVENSTER, 1961). However, the number of ripe female test fish on a certain day was often limited and therefore sub-optimal females had to be used sometimes in courtship tests.

In general repeatabilities can be considered as the upper limits to heritabilities of the same traits under the same conditions. Strictly speaking we should concentrate on the repeatabilities of the various aggression scores, since the heritabilities that will be estimated from the selection experiments (see chapter 3) will be based on aggression scores rather than single test results. It should be expected that these repeatabilities will achieve even higher values, because scores are defined as mean values of repeated measurements.

In the next section I apply another method to estimate heritabilities: the full sib correlation. In principle, a comparison of the variation between and within full sib families yields more accurate estimates of heritabilities than would be obtained from repeatabilities (see section 2.1.4.).

2.6.2. *Full sib correlation.*

The sharing of genes is one cause of the resemblance of relatives. In the case of full sibs (which have both parents in common, and consequently have a chance of .25 of having the same genotype for any locus) not only the additive genetic variance but also the dominance variance contributes to the resemblance. Sharing of common environments can be another cause of resemblance between relatives, especially between full sibs. However, in the present study all fish were isolated at an early age (see section 2.1.2.), thus precluding as much as possible the environments of relatives from being more similar than those of non-relatives, thus reducing this possibility as a cause of resemblance between related fish.

The base population was set up for artificial genetic selection and was therefore made as heterogeneous as possible by chosing only a low number of full sibs for each family (see n_{fs} in Table 14). This is somewhat contrary to the aim of estimating heritabilities from full sib correlations, since in the most efficient design with full sib families a family size of $2/(h^2)$ is required (FALCONER, 1981).

The analyses of variance for the full sib correlations have been carried out with the most reliable aggression scores (as for the phenotypic correlations, scores based on less than 3 test results—or in juvenile tests less than 4—were omitted from analyses). The resulting heritability estimates (Table 14), though not statistically significant due to small family sizes, are consistent with the repeatability values of Table 12: they cover about the same range, but do not exceed them.

TABLE 14. Heritability estimates for aggression during the different behavioural tests, based on full sib correlations in the base population

Aggression score	n_p	n_{fs}	t_{FS}	F	p	h^2_N
JAS (♂♂)	7	2.27	0.13	1.34	ns	0.26
JAS (♀♀)	14	2.49	0.21	1.67	ns	0.43
JAS (♂♂ + ♀♀)	21	3.27	0.22	1.95	<0.05	0.45
FAS	15	2.65	0.24	1.83	ns	0.48
TAS	9	2.54	0.29	2.04	ns	0.58
CAS	9	2.54	0.26	1.89	ns	0.52

n_p = number of parental pairs, n_{fs} = number of full sibs (weighted average), t_{FS} = full sib correlation, F = variance ratio, p = probability, h^2_N = rough estimate of the heritability in the narrow sense, based on $2t_{FS}$. For aggression score abbreviations see Table 11.

The only reliable heritability estimate based on full sib correlation is that for juvenile aggression scores of both males and females. Such a calculation can only be justified if juvenile aggression scores of both sexes

have an identical gentic basis. Indications for this are presented in this chapter (in so far as one can judge from phenotypic similarities; see sections 2.2. and 2.5.) and extended in the next chapters.

The picture that has emerged from the analysis of aggressiveness in the base population is elaborated in the next chapter (ch.3), where the various aggression scores are used as criteria for artificial selection for both enhanced and reduced aggressiveness.

2.7. Summary.

Progeny of wild-caught three-spined sticklebacks (*Gasterosteus aculeatus* L., forma *leiura*), isolated at an early age in small tanks, formed the base population of a selection-program for aggressiveness. This base population can be looked upon as a representative sample of a natural population. In this chapter the variation of aggressiveness in the base population is analysed. Aggressiveness is measured in 5 different situations with the aid of standardized tests, viz. juvenile, female, male, courtship and dominance tests. In the three first named tests the experimental fish (juvenile, female in reproductive condition, male in reproductive condition) were confronted in their own tanks with an opponent of the same age and sex, enclosed in a tube or chamber. In the courtship test the behaviour of a territorial male upon exposure to an enclosed ripe female was recorded. Finally, in the dominance test the dominance relation of two isolated males in reproductive condition was assessed in a tank that was unfamiliar to both males.

It is shown that in each test situation aggressiveness, expressed as the mean duration of biting and bumping against an opponent, is highly variable across individuals in the base population.

Juvenile aggressiveness declines gradually with age. Males and females do not differ with respect to their mean juvenile aggressiveness. The transition to sexual maturity is not accompanied by a change in mean aggressiveness in the females. However, the mean aggressiveness of males increases considerably once they reach sexual maturity and have built a nest.

Further, isolated territorial males can be arranged in a linear order of dominance, when tested pairwise in dominance tests. The rank in such a dominance order is not correlated with the levels of aggression during the other tests. In males only territorial and courtship aggressiveness are correlated to some extent. In females aggressiveness during the juvenile and adult stages shows a rather high phenotypic correlation coefficient. Dominance tests with adult females generally produce less clear-cut results than those with males, though in some tests clear dominance relationships are established and even roundabout fights are occasionally observed.

The part of genetic variance in the total phenotypic variance of aggressiveness in the different test situations is estimated from both the similarity of repeated tests with the same individuals and from the resemblance of full sibs with respect to their aggressiveness. The estimates thus obtained, which represent the upper limits of heritabilities for aggressiveness in the situations concerned, are rather high and therefore predict clear responses when aggressiveness in these test situations are going to be used as criteria for artificial directional selection.

3. Selection for aggressiveness

3.1. Introduction.

This introduction briefly treats the different approaches for the genetic analysis of behaviour in section 3.1.1., with the emphasis on the method

that is applied in the present study, *i.e.* directional selection. Next, section 3.1.2. shows the obvious gap in our knowledge of fish behaviour in reviewing behaviour genetic studies on aggressiveness in fish.

3.1.1. *Directional selection.*

In general, there exist three different approaches for the analysis of the genetic architecture of quantitative, behavioural traits. The choice of the approach depends, among other things, on the availability of groups of individuals that differ in the behavioural trait one is interested in, such as inbred lines, selected lines, races or related species. If these are available then the most plausible approach is to make crosses between such groups according to standardized crossing schemes (viz. Mendelian cross, diallel cross, and triple-test cross). In fact, this approach is the most powerful one for unravelling the genetic architecture of behavioural traits, especially when a number of inbred lines is used (see *e.g.* MATHER & JINKS, 1982; JINKS & BROADHURST, 1974).

When inbred lines are not available two different approaches exist for the genetic analysis of behaviour. One is based upon the resemblance between individuals of different degrees of relationship. This approach is particularly useful if the relationship of individuals is known and the possibility of selective breeding restricted or precluded, as in farm animals and humans (see *e.g.* FALCONER, 1981; MCCLEARN & DEFRIES, 1973; BODMER & CAVALLI-SFORZA, 1976).

The other approach when inbred lines are lacking is artificial selection, and more specifically directional or linear selection (MATHER, 1953). It involves starting with a heterogeneous base population and in each successive generation chosing individuals at one extreme of the distribution of phenotypic values as parents for the next generation. Under the assumption of a polygenic mode of inheritance of the behavioural trait in question with many alleles increasing or decreasing performance, directional selection effects the concentration of increasing alleles in the line selected in upward direction and of decreasing alleles in the one selected in downward direction.

Directional selection provides the most obvious evidence for the contribution of genetic variation (more specifically additive variation) to the phenotypic variation, and is useful for estimating heritability values (in the narrow sense). Selection experiments have not been designed to unravel the genetic architecture of the behavioural trait under selection pressure in great detail, but the resulting selection lines are very suitable for further behaviour genetic analysis (as illustrated by the genetic

analyses with selection lines *e.g.* for geotaxis in *Drosophila melanogaster*; HIRSCH, 1959, and subsequent papers, for phototaxis and geotaxis in *D. pseudoobscura*; DOBZHANSKY & SPASSKY, 1967, and subsequent papers, and for locomotor activity in *D. melanogaster*; VAN DIJKEN & SCHARLOO, 1979, and subsequent papers).

On the other hand, knowledge about natural selection may be deduced from the limitations of artificial, directional selection. When a trait has been subject to long-term directional selection, the additive genetic variation will be depleted. The remaining genetic variation will be dominance (interaction between alleles) and interaction (interaction between different genes) variation. If this situation has occurred as a consequence of natural selection, artificial two-way selection should be expected to result in asymmetrical responses (*e.g.* FALCONER, 1981; BROADHURST & JINKS, 1974). Such a genetic architecture and the consequent asymmetrical responses to bidirectional selection are characteristic for characters which are closely connected to fitness. Artificial selection towards increased fitness will give a slower response than selection towards decreased fitness. The genetic architecture of characters which have been subject to long-term stabilizing selection is characterized by a large additive component of variation relative to the size of the dominance component. For such traits responses to artificial bidirectional selection should be expected to be symmetrical (*e.g.* FALCONER, 1981; BROADHURST & JINKS, 1974). There are, however, many possible causes for asymmetrical responses to bidirectional selection (see section 4.4.) and the cause operating in a particular case is hard to identify. Therefore, conclusions about natural selection which are drawn from responses to artificial two-way selection must be considered tentative without knowledge of the genetic architecture of the trait involved.

3.1.2. *Behaviour genetic studies on aggressiveness in fish.*

Most behaviour genetic research is concerned with species, such as mouse (*Mus musculus*), rat (*Rattus norvegicus*) and fruitfly (*Drosophila melanogaster*), of which inbred strains are readily available (see *e.g.* EHRMAN & PARSONS, 1976). Behaviour genetic research with fish is less common despite the fact that there are fish species of which laboratory inbred strains exist (*e.g.* platyfish and swordtails, all belonging to the genus *Xiphophorus*; KALLMAN, 1975). One of the few extensive behaviour genetic studies with fish focuses on the courtship behaviour of hybrids of *Xiphophorus* (FRANCK, 1970, 1974), but if we focus our attention on aggression in fish yet fewer studies are available.

So far, three fish studies have been published in which crosses were used for the genetic analysis of aggressiveness. PARZEFALL's (1979) analysis of the aggressive behaviour of two populations, *i.e.* an epigeous and a hypogeous (cave-dwelling) population, of the toothcarp *Poecilia sphenops*, and their hybrids (F1, F2 and backcrosses) revealed that the reduction of aggressive behaviour among males in the cave population was genetically controlled. A second study made use of two inbred strains of guppies (*Poecilia reticulata*) with very different quantitative sexual behaviour patterns, and their crosses (F1s and backcrosses). A simple analysis of the proportion of tanks (2 ♂♂ and 2 ♀♀) in which intermale aggression was observed, showed a strong Y-linkage of this difference in aggressive behaviour between the two strains (FARR, 1983). Lastly, a behaviour genetic study on differences in aggressiveness between two closely related fish species was carried out by FERGUSON & NOAKES (1982, 1983). Juvenile brook charr (*Salvelinus fontinalis*) are territorial and have high levels of aggressive behaviour, while juvenile lake charr (*S. namaycush*) are non-territorial and rarely show any aggressive behaviour. These differences were summarized in a compound score, low scores indicating high aggression. The distribution of scores for the crosses (F1s, F2 and backcrosses) clearly showed the genetic involvement in the behavioural differences between brook and lake charr. A significant maternal effect was assessed in the phenotypic expression of behaviour in these fish and the results further suggested directional dominance in favour of the more aggressive phenotype.

Detailed behaviour genetic analyses of aggressiveness based on the resemblance between relatives have not been published for fish. Of course there are numerous comparative studies in which the aggressiveness of different related species or different populations of one species is quantified. However, even if the fish were reared under comparable circumstances, differences in aggressiveness between populations or between species are at the utmost indicative for genetic variation, but can never be conclusive without further genetic analysis. An example is a study on behavioural individuality in the cichlid fish *Tilapia mossambica* by BARASH (1975). He arranged males derived from different sources into a few classes according to their aggressivenss towards a male model. Their offspring showed a remarkably similar degree of aggressiveness. Also GOYENS & SEVENSTER (1976) found large differences in juvenile aggressiveness between groups of isolated offspring from different parents of the three-spined stickleback, kept under standardized conditions.

There exists but one study in which aggressiveness in fish was chosen as a criterion for artificial selection. This recently published study by FRANCIS (1984) concerned bidirectional selection for social dominance in the paradise fish (*Macropodus opercularis*). After two generations of selection the two selection lines deviated significantly in their dominance success. Several results of this study run parallel to those of the present study. These will be discussed in the relevant sections, and in section 3.6. this selection experiment will be compared with studies in other vertebrates involving selection for aggressiveness.

This review of genetic studies on aggressiveness in fish, shows the obvious gap in our knowledge of fish behaviour. Even in a well-known species, like the three-spined stickleback, behaviour genetic studies are limited to that of SEVENSTER & 't HART (1974) on "double creeping through". In this study, where directional selection and classical Mendelian crossing schemes were applied, an aberration in males with respect to creeping through could be ascribed to a major genetic factor that showed simple Mendelian inheritance, with the normal condition being dominant.

As a continuation of his study on territory in the three-spined stickleback, VAN DEN ASSEM 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 present study was set up to investigate, among other things, this topic in greater detail.

3.2. Material and methods.

3.2.1. *Selection lines and control line.*

From the base population (generation 0) described in the previous chapter, six different selection lines were founded. This means that, for each line, males and females of the base population with the most extreme scores for one of the tests were mated, according to the scheme presented in Fig. 11 (but notice the dominance lines). Part of their progeny was isolated again and formed the first selected generation (generation 1) of the various selection lines. The testing procedure was repeated in this generation and again, within each selection line, parents were selected according to the criteria of their line (Fig. 11), and mated, *etc.* In total, this procedure was repeated four times, so that 5 generations (the base population and 4 selected generations) were involved. The last generation was used in another project (see chapter 4). The various lines will be indicated from now on by the abbreviations given in Fig. 11, to which, if necessary, a generation number is added.

The numbers of animals used for these selections are presented in the appropriate tables. In each selection line and in every generation we aimed at breeding three parental pairs. As a rule these parental pairs were used once, if the particular combination produced enough progeny. Inbreeding was avoided as much as possible in order to reduce the influence of genetic drift.

Selection Line	Abbr.	Criterion of Selection ♂♂	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 (D)	Dominance Ability (D) of their brothers
Control Line	C	—	—

Fig. 11. Selection lines and the criteria upon which selection was based. H = high line, L = low line.

In selecting potential parents, only the aggression score relevant for the selection line in question was taken into account. On the other hand, each isolated fish of all selection lines was submitted to all the behavioural tests mentioned in the previous chapter (remember Fig. 2), enabling us to study the effect of the selection in each line on the other scores (see chapter 4: correlated responses). In the present chapter we focus on the changes of the aggression scores directly selected for.

About 10 young chosen at random from the progeny of each parental pair were isolated individually to form the next generation; thus with three parental pairs and a sex-ratio of one, within every selection line about 15 females and 15 males were tested in each generation. Another part of the progeny was retained as groups of full sibs, and used in another project (see chapter 4).

From the base population (C-0) different parents were selected to found each of the various selection lines. The remaining, unselected C-0 fish did certainly not form a representative sample of the population studied. Parents to produce generation 1 of the control line were therefore randomly chosen among full sibs (held in groups) of the isolated individuals. These spare groups, each produced by different parents, consisted of 15 full sibs, and had been sampled from the same fry as the isolated C-0 fish. In following this procedure the implicit assumption was made that the isolated fish and their full sibs in groups did not differ genetically. In every generation the control line consisted of the progeny of about 10 parental pairs that were randomly chosen from the previous generation. In generation 2 juveniles of the control line (C-2) were again isolated and tested. C-4 juveniles were also isolated to be used in another project (see chapter 4).

In raising the base population wild-caught sticklebacks were bred in tanks of 34.5 × 40 × 50 cm and the males left in charge of the developing clutches. After generation 0 breeding was carried out in the small tanks of isolated males, but about one hour after fertilization, when the clutches have hardened sufficiently, they were carefully transferred to plastic trays filled with tapwater and aerated through an airstone. In spite of occasional fungal infections of the clutches, this artificial hatching system facilitated breeding. Five to 7 days after fertilization clutches were analysed microscopically: eggs were counted and any deviations from normal development recorded. When all eggs of a clutch had hatched (about 8 days after fertilization), the fry was transferred to a small standard tank (Fig. 1). Here they remained until isolation at day 21 after fertilization (about two weeks earlier than for C-0 fish).

The behavioural test schedules were similar to those described in section 2.1.2. except for a few alterations mentioned below.

Juvenile tests.

The isolated juveniles were first submitted to the juvénile test at an age of 35 days after fertilization, a week earlier than in C-0. The test frequency was reduced to one juvenile test each two-week interval. Because of time limitations this was necessary since the total number of young was twice that of C-0. If juveniles did not reach the test-tube within the available 5 minutes after its introduction, the invalid test was repeated a week later.

Dominance tests.

In each generation in both dominance lines there were fewer males than in C-0. It therefore became feasible to subject all combinations of two males within both lines to a dominance test in order to arrange the males within the DH and DL line in an order of dominance. Occasionally the tests remained "undecided" for a long time; the pairs were then observed for at least 15 minutes, but usually much longer. The determination of the reaction to selection (response) of the dominance lines poses specific problems, as outlined in the next section.

3.2.2. *Response in dominance lines.*

To assess the response to selection the mean dominance ability of successive generations of the dominance lines should be compared. However, because dominance is a relative measure, this comparison cannot be made directly.

One possibility is to compare, for each generation, dominance abilities of males of the dominance lines with those of the control line. This has been done only in generation 2, where isolated C males were available; a sample of 10 randomly chosen combinations of males of each line and males of the C line were submitted to a dominance test.

Another possible method to determine the response to selection for dominance ability is to assess, for each generation, the difference in mean dominance ability between the high and low line. For that purpose a number of dominance tests between lines (varying between 10 and 20) were performed for each generation, using randomly chosen males of both lines. The response to selection for dominance, measured from the divergence of the two lines, can be expressed in two ways:

a. Deviation from the 50%-level (equality of mean dominance ability) of winning the tests by DH or DL males, and

b. Degree of non-overlap between both linear orders of dominance.

The latter measure needs some further explanation. The results of the dominance tests between males of the two dominance lines can be used to construct a combined order of dominance. Thus, the linear orders of dominance of both separate lines, as determined before, may be partly intertwined. If we assume that the combined order of dominance is also linear (this assumption is justified by the test results, see section 3.5.1.), then the degree of non-overlap between the two orders of dominance can be expressed as the mean difference between the two mean ranks in a combined order of dominance. Thus, this can be expressed in the following equation:

$$NO = \frac{\dfrac{\sum_{i=1}^{n_H} D_{Hi}}{n_H} - \dfrac{\sum_{i=1}^{n_L} D_{Li}}{n_L}}{n_H + n_L}$$

where NO = degree of non-overlap, D_{Hi} = rank of the i-th male of the DH line in a combined order of dominance, D_{Li} = rank of the i-th male of the DL line in a combined order of dominance, n_H = number of DH males, n_L = number of DL males.
The value of NO is independent of the number of males involved, and can vary between 0 and 0.5 (the most inferior male is given the lowest rank). When, in a given generation, the two dominance lines are identical with respect to their mean dominance ability, their overlap will be maximal, and their non-overlap minimal (*i.e.* 0), because of identical mean ranks of both lines. When overlap is minimal and non-overlap is maximal, as is the case when the least dominant male of the high line is more dominant than the most dominant male of the low line, it can be easily shown that NO will be 0.5 in that case.

Time limitations restricted the number of dominance tests between lines, resulting in some uncertainties in the precise succession of male's combined order of dominance. Therefore two estimates of NO were made: one based on highest ranks for DH males in case of doubt (DH favoured NO, which is likely to overestimate NO), and one based on highest ranks for DL males in case of doubt (DL favoured NO, which is likely to underestimate NO). The actual value of NO will lie somewhere between both extreme values of NO. As an example the position of male K may be presented. Given the three low line males E', F' and G' in the sequence of decreasing dominance, and the test outcomes: K of the high line dominates G', but E' dominates K, then the two extreme orders are: E' K F' G' if DH is favoured (that is, K is *assumed* to dominate F') and E' F' K G' if DL is favoured (that is, F' is *assumed* to dominate K).

3.2.3. *Colouration.*

On various occasions the colouration of the males was quantified by the following four-point scale:
1. Bright: intensily red, practically no black pigmentation.
2. Fair: much red on throat and ventral region. Caudal region with more or less pronounced black pigmentation.
3. Moderate: some red on throat only.
4. Dull: no sign of red.

Thus the higher his colouration score, the duller the colouration of a male in reproductive condition.

3.2.4. *Methods of quantitative genetics.*

In selection experiments heritabilities, in the narrow sense (h^2), can be estimated from the ratio of the response (R) to the selection differential (S):

$$h^2 = \frac{R}{S}$$

where R = the difference in mean phenotypic value between the offspring of the selected parents and the parental generation, S = the mean phenotypic value of the individuals selected as parents, expressed as a deviation from the population mean.

Heritability estimates from selection experiments are obtained by plotting the generation means against the cumulative selection differential. The slope of the regression line through these points measures the average value of R/S, which is called the realized heritability.

To account for the variable numbers of offspring that are tested per parental pair the selection differentials are weighted by the following equation:

$$\text{weighted } S = \frac{1}{n} \sum \left[\frac{f_i}{\bar{f}} (X_i - \bar{X}) \right]$$

where f_i = number of progeny produced by the i-th parental pair, $\bar{f}$ = average contribu-

tion of all parental pairs, X_i = midparental value of the i-th parental pair, $\bar{X}$ = generation mean of midparental values, n = number of parental pairs.

In the above treatment of selection it is supposed that the quantitative trait under study can be measured in the same way in both males and females. In the present study this is not always possible. In the territorial aggression lines, the males are selected according to their territorial aggression score, and the females according to their female aggression score. Because these aggression scores differ considerably among sexes, heritability estimates were calculated for both sexes separately, by comparing aggression scores of fathers with those of their sons, and scores of mothers with those of their daughters. The slopes of the corresponding regression lines are multiplied by 2 in such one-parent offspring comparisons to yield accurate h^2-estimates (FALCONER, 1981). This doubling of the regression coefficient is, however, only justified if mating is random. In the case of assortative mating, which can be measured by the correlation coefficient (r) of the two parents, this doubling is reduced by dividing the regression coefficient by a factor (1+r) (CAVALLI-SFORZA & BODMER, 1971).

These difficulties in the h^2-calculations can be avoided if one parent is selected according to a certain criterion and the parent of the opposite sex chosen at random with respect to this criterion. In the present study a course was adopted in which both sexes were selected, in the expectation that clear responses within a limited number of generations will be obtained. This implies that in both sexes the genetic basis for the aggression score is, at least partly, identical, a questionable assumption. Do females, selected for a high female aggression score, contribute to a high territorial aggression score of their sons? An operational approach to such questions would be to compare the regression of the selection response to the cumulative selection differential in one sex with the regression of the same selection response on the selection differential of the other sex (SCHARLOO, pers. comm.). This cross-regression method will be applied in the sections 3.3.2. and 3.4.2., where it will be shown that females do contribute to the scores of their sons, and males to the scores of their daughters. Whether this contribution is identical for both sexes remains uncertain. But for the calculations we will assume that this is so, so that the slopes of the R-S regression lines can be taken as estimates of the various h^2-values. It should be realised, however, that if this is not the case, the h^2-values are underestimated.

The estimation of heritabilities requires normally distributed variables. For juvenile and territorial aggressiveness such an assumption seems to be justified by the frequency distributions shown in the previous chapter. In the case of adult females we have to be cautious because of the skewed distribution of female aggression scores. Dominance ability certainly does not fulfil this demand, and as a consequence h^2-calculations for dominance ability pose specific problems (see section 3.5.2.).

3.3. Juvenile aggression lines.

In this section 3.3. the results of selection for juvenile aggression are treated in terms of the "% biting-bumping time in the tube-period" in a juvenile test, and its mean value during the tests prior to week 15 after fertilization (which is called the juvenile aggression score or JAS, see section 2.2.). The changes in other measures of juvenile aggressiveness and in aggressiveness in other test situations, that accompany the selection for JAS (*i.e.* correlated responses), will be dealt with in the next chapter (chapter 4). Here our primary concern is the change of JAS under bidirectional selection pressure. The selection responses of both juvenile

males and juvenile females are presented in section 3.3.1. In section 3.3.2. heritability-values for juvenile aggressiveness are estimated. And finally the course of juvenile aggressiveness as a function of age is subjected to a closer examination in section 3.3.3.

3.3.1. *Responses.*

Selection for a reduced juvenile aggressiveness has been highly successful in both juvenile males and juvenile females (Fig. 12). Even in the first generation of selection the difference in JAS of either sex with the base population is significant (Mann-Whitney U test, 1-tailed: juvenile males $p<0.05$, juvenile females $p<0.01$). In the succeeding generations these differences remain highly significant ($p<0.01$ for both sexes).

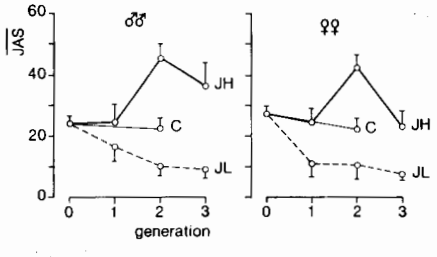

Fig. 12. Responses of the juvenile aggression lines. $\overline{JAS}$ = mean juvenile aggression score. Lengths of bars represent one standard error of the mean.

Selection for an increased juvenile aggressiveness appears less effective. Although a significant increase in juvenile aggressiveness had been established in both sexes after two generations of selection ($p<0.01$ relative to both C-0 and C-2), juvenile aggressiveness dropped to the control level in the next generation, sharply in females and less so in the males. Even in the juvenile males the distribution of juvenile aggression scores of JH-3 and C-0 (or C-2) are not significantly different ($p>0.05$) (Fig. 13).

A possible cause of this sudden decline in juvenile aggressiveness in JH-3 is limitations in our breeding schedule. In section 4.4. we will return to this problem, but here we deal with one of the possibilities, viz. the selection of females for breeding.

On the basis of their juvenile aggression scores five females from JH-2 were selected as potential female parents. However, two of these females failed to breed. One failed to spawn on 7 different days she was ripe. The

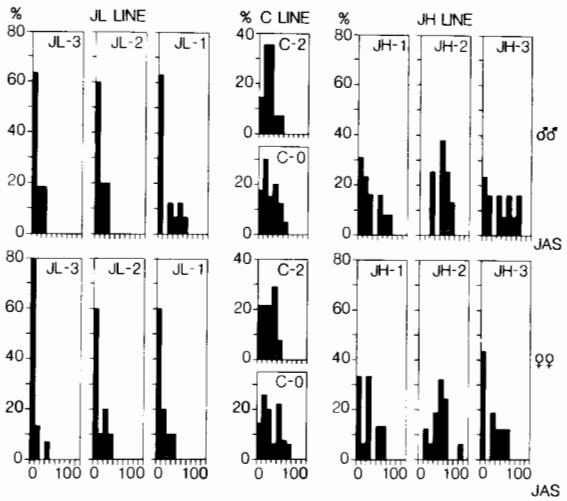

Fig. 13. Frequency distributions of juvenile aggression scores (JAS) of the JL, C and JH line.

other consistently attacked the courting male on 10 different days she was ripe. Often the male then counter-attacked, and these attempts to breed thus resulted in intense roundabout fights. On one occasion the male even fled from this she-man. Thus, three females were left as potential parents. Together they produced four clutches with a mean clutch-size of 143.3 eggs. In one of these clutches (the one produced by the female with the highest JAS in JH-2) the development of the embryos was strongly retarded. Only 7 young reached the age of 21 days (after fertilization) at which time they were isolated. It was decided to include them in generation JH-3 to preserve the full genetic variation. The juvenile aggression scores of these young, however, appeared to be very low (on the average 7.5% for both juvenile males and females). In this particular case selective embryonic death seems to be the most plausible inference. If these young are excluded from the calculations, then the mean JAS of juvenile males of JH-3 shifts from 36.5% to 41.8% and becomes significantly different from the distribution of C-0 and C-2 at the 5% level. The mean JAS of the juvenile females shifts from 23.0% to 28.2%, but remains not significantly different from the control line. In summary, limitations in spawning and selective embryonic death cannot be ruled out as possible causes for the decline in mean juvenile aggressiveness of the JH line in generation 3.

3.3.2. *Heritabilities.*

The result of further analysis of selection for juvenile aggressiveness is presented in Table 15. Because the mean JAS in the control line declines slightly, but not significantly, for both juvenile males and juvenile females, cumulative responses have been related to the mean JAS of C-0 and C-2.

The decrease of JAS in the control line may be due to the somewhat different age at isolation and age at the first juvenile test in C-0 (see section 3.2.1.). If the outcomes of the first juvenile tests for C-2 juveniles are ignored, then the mean score shifts to 24.0% for the juvenile males and to 25.4% for the juvenile females, indicating the influence of the first (added) test of C-2 juveniles.

The selection differential in the high line appears to be nearly twice as large compared to the low line (see Table 15). This, however, is a misrepresentation of the applied selection pressures, which were comparable for both lines in terms of the proportions selected in each generation, and is largely due to scale-effects. These scale-effects are caused by changes in the variation of JAS in the same directions as the changes in the generation means. Thus, the variation in the low line is reduced compared to the high line, as is evidenced by the higher coefficient of variation (*i.e.* SD corrected for the mean) of the low line compared to the high line.

To compare selection differentials one ought to express them in terms of the phenotypic standard deviation. This standardized selection differential is called the intensity of selection, and depends only on the proportion of the population included in the selected group, which in our case does not differ much between both selection lines.

From the figures of Table 15 realized heritabilities have been calculated for JAS in the JL line (Table 16). These h^2-estimates appear to be rather high, indicating a large amount of additive genetic variation for low juvenile aggressiveness in the base population for both juvenile males and juvenile females. The present study unfortunately does not allow a reliable estimate for the realized h^2 of juvenile aggressiveness in the high line, as is evident from the previous section.

The regression of cumulative response on cumulative weighted selection differential yields roughly the same slopes whether applied to one sex or both sexes of the low line (difference between h^2 of either sex and h^2 of both sexes together is not significant: t test, $p > 0.50$), pointing to an equal contribution of both sexes to the juvenile aggression scores of their progeny.

TABLE 15. Results of selection for increased (JH line) and decreased (JL line) juvenile aggressiveness

	JH Selection Line						C Line	
Genera-tion	Tested ♂♂ n	$\overline{JAS}$	Selected ♂♂ n	$\overline{JAS}$	Cumulative R	Cumulative weighted S	Tested ♂♂ n	$\overline{JAS}$
0	40	24.2	4	39.4	0.0	0.0	40	24.2
1	13	24.1	2	49.4	+ 0.7	+13.6	—	—
2	8	45.6	2	59.2	+22.2	+38.9	14	22.6
3	13	36.5	1	71.4	+13.1	+51.9	—	—
	Tested ♀♀		Selected ♀♀				Tested ♀♀	
0	55	27.5	4	46.7	0.0	0.0	55	27.5
1	15	24.4	4	43.6	- 0.4	+17.2	—	—
2	16	42.4	2	67.8	+17.6	+37.5	14	22.1
3	16	23.0	1	42.9	- 1.8	+55.9	—	—
	Tested juveniles		Selected parents[a]				Tested juveniles	
0	95	26.1	5	41.9	0.0	0.0	95	26.1
1	28	24.2	4	46.5	- 0.1	+14.9	—	—
2	24	43.4	3	62.3	+19.1	+36.7	28	22.4
3	29	29.1	1	57.2	+ 4.8	+53.5	—	—

	JL Selection Line						C Line	
Genera-tion	Tested ♂♂ n	$\overline{JAS}$	Selected ♂♂ n	$\overline{JAS}$	Cumulative R	Cumulative weighted S	Tested ♂♂ n	$\overline{JAS}$
0	40	24.2	4	10.8	0.0	0.0	40	24.2
1	16	16.7	1	7.3	- 6.7	-12.1	—	—
2	10	10.0	2	2.0	-13.4	-21.5	14	22.6
3	11	9.3	3	1.5	-14.1	-29.3	—	—
	Tested ♀♀		Selected ♀♀				Tested ♀♀	
0	55	27.5	4	9.2	0.0	0.0	55	27.5
1	10	11.0	1	11.5	-13.8	-19.7	—	—
2	10	10.5	3	1.1	-14.3	-19.2	14	22.1
3	15	7.4	3	1.3	-17.4	-28.5	—	—
	Tested juveniles		Selected parents[a]				Tested juveniles	
0	95	26.1	5	10.2	0.0	0.0	95	26.1
1	26	14.5	1	9.4	- 9.8	-16.2	—	—
2	20	10.2	3	1.2	-14.1	-21.3	28	22.4
3	26	8.0	4	1.7	-16.3	-29.8	—	—

$\overline{JAS}$ = mean juvenile aggression score ([a] = mean mid-parent JAS), R = response in regard to mean of C-0 and C-2, S = selection differential, C = control line, n = number of fish [a] = number of parental pairs).

TABLE 16. Realized heritabilities (h^2) for JAS in the JL line, as estimated from the regression of the selection response on the cumulative selection differential

Type of selection	h^2	s_b	r	p
JL ♂♂	0.51	0.08	0.98	$0.01 < p < 0.025$
JL ♀♀	0.64	0.07	0.99	$0.005 < p < 0.01$
JL ♂♂ + ♀♀	0.57	0.06	0.99	$0.005 < p < 0.01$

s^b = standard deviation of the regression coefficient, r = correlation coefficient, p = probability.

Another method of checking the validity of the above assumption is to calculate cross-regression lines by plotting the response in one sex against the applied selection differential in the other sex (see section 3.2.4.). If these cross-regression lines fit as well as the ones obtained by the standard procedure (like in Table 16), then the share of both sexes in the juvenile aggressiveness of their progeny is likely to be the same. The results of this analysis, summarized in Table 17, fail to reveal a sex-linked difference in the contribution to juvenile aggressiveness. The difference between the correlation coefficients with normal- and cross-regression do not deviate significantly from 0 ($p > 0.70$, for both cases), although the cross-regression lines fit slightly less well (in both cases, s_b is somewhat larger with roughly equal values of the slopes, and r is somewhat lower). Because juvenile aggression scores of males and females have about the same range and because the applied selection differential have been roughly equal in both sexes, the slopes of the cross-regression lines correspond to the realized heritabilities (differences between the regression coefficients with normal- and cross-regression do not significantly deviate from 0: t test, $p > 0.50$, for both cases).

TABLE 17. Cross-regression for JAS in the JL line

Type of regression	b_{RS}	s_b	r	p
R(JL ♂♂) - S(JL ♀♀)	0.50	0.17	0.90	$0.025 < p < 0.05$
R(JL ♀♀) - S(JL ♂♂)	0.55	0.17	0.92	$0.025 < p < 0.05$

For explanation see text. R = response, S = selection differential, b_{RS} = regression coefficient of R on S. For remaining symbols see previous table.

The realized heritabilities for JAS in the JL line are rather high even if compared with the h^2-estimates gained from the full sib correlations (cf. Tables 14 and 16). This suggests an asymmetry of the response to selection in the high and low selection line. In the JH line a significant response was lacking after three generations of selection, while in the JL line a distinct response was obtained. Whether this asymmetry of response would exist after persistent selection remains to be seen. If so, then a discrepancy is expected between the h^2 estimated from the full sib correlation in the base population and that estimated from the selection response in one of the two directions, since in that case the h^2 estimated from the former would presumably correspond best to the mean value of the realized heritabilities in the two directions.

3.3.3. *Changes in aggression as a function of age.*

The mean course of juvenile aggression as a function of age in the successive generations of the JH, JL and C line is presented in Figs 14 and 15 for juvenile males and juvenile females, respectively. Juvenile aggressiveness shows a rather characteristic course with age; in the first

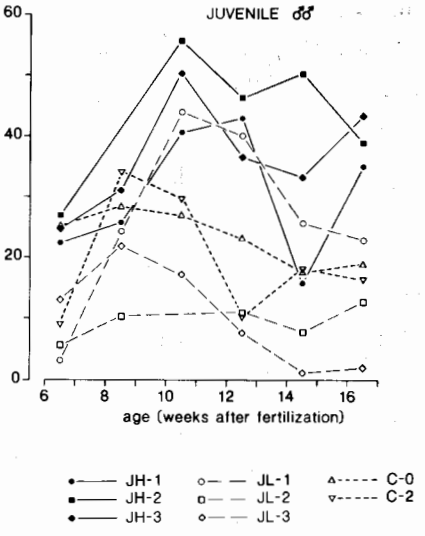

Fig. 14. Mean juvenile aggressiveness as a function of age in juvenile males of the JH line, the JL line and the C line.

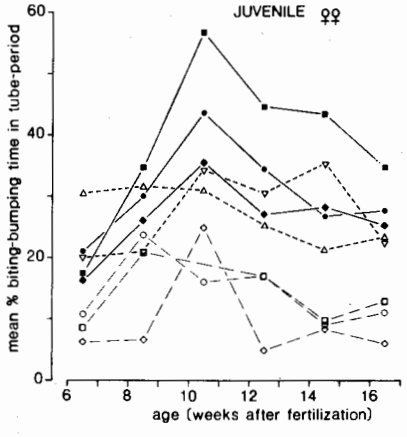

Fig. 15. Mean juvenile aggressiveness as a function of age in juvenile females of the JH line, the JL line and the C line. Legends as in Fig. 14.

month or so after its onset juvenile aggressiveness increases with age and then declines until sexual maturity. The period of increase of juvenile aggressiveness is more prolonged in the JH line than in the JL line, and as a result the peak-position is different in both lines. The juvenile aggressiveness of males in the JH line remains high after the peak for several weeks, and even increases again in the 16-17th week after fertilization in generation JH-1 and JH-3. This higher level of juvenile aggression of males prior to nestbuilding probably corresponds to the level of aggression often termed pre-breeding, pre-nest or pre-spawning aggression in the literature (*e.g.* WOOTTON, 1970; HUNTINGFORD, 1979).

The general decline of juvenile aggressiveness with age after the peak-values cannot be ascribed to habituation to the test situation: the mean level of juvenile aggressiveness in the base population shows a comparable degree of decline with age, whereas the test frequency there was twice that of later generations (see also section 4.6.1.).

Compared to C-2 and the various generations of the juvenile aggression lines, the base population's course of mean juvenile aggressiveness with age shows less variation and starts at a higher level. This is due to the somewhat different test schedule in C-0 (see section 3.2.1.). Compared to the generations after C-0, in the base population more individuals were tested, and with double the test frequency. In Figs 14 and 15, therefore, the test results per C-0 juvenile are averaged for two successive weeks. Although the overall responsiveness in the juvenile tests was comparable for the JH, JL and C line, the proportion of non-responders in each age-class was quite variable and sometimes greater in the JH and JL line and in C-2 than in C-0. The net result is that, compared to the generations after C-0, the course of mean juvenile aggressiveness in C-0 is based on more test results. Furthermore, testing in C-0 was mostly begun a week later than in the later generations. Therefore the first plotted point (mean of week 6 and 7) of C-0 in Figs 14 and 15 includes only few juvenile tests in week 6. Since juvenile aggressiveness increases from week 6 to 7, this first plotted point gains relatively too high a value in C-0, as is easily demonstrated by omitting test results of week 6 in C-2 juveniles. By doing so, the first plotted point of C-2 increases to 23.2% for juvenile males and to 29.0% for juvenile females, thus matching the first plotted point of C-0.

Extrapolation of Figs 14 and 15 to the age of onset of juvenile aggressiveness suggests that the first signs of juvenile aggressiveness will be earlier in the JH line than in the JL line. This suggestion is supported by the fact that aggression in groups of JH-3 juveniles started about a week earlier than in groups of JL-3 juveniles (see section 4.6.1.). This discrepancy in the onset of aggressiveness might reflect a difference in the rate of general development between juveniles of the JH and JL line. Some evidence for this hypothesis will be presented below.

As stated earlier (section 2.1.1.) C-0 juveniles were isolated at the first sign of aggression in the groups. These groups, consisting of the progeny of wild-caught parents, were kept in tanks of 34.5 x 40 x 50 cm; the fathers were removed a few days after hatching.

Daily checks on the onset of aggression suggested that the groups differed in this respect: the first sign of aggression occurred at a mean age of 34.7 days after fertilization, with extremes of 28 and 45 days. This variation (y) could be related to the variation in group-size (x): $\hat{y} = 0.064x + 29.91$, $r = 0.44$, $0.02 < p < 0.05$ (t test, 2-tailed, df 23). This unexpected relationship suggests a retardation in development with increasing group-size, which could be explained by the smaller amount of food per juvenile with increasing group-size. Groups were fed twice a day, and small groups may well have had relatively more food than large groups, or at least the food may have been better available to all individuals. Further evidence for this retardation in development with increasing group-size is obtained from the variation in the age of sexual maturity (♂♂: completion of the first nest, ♀♀: readiness to spawn for the first time) in their isolated full sibs (the base population). In juvenile males there is a clear-cut correlation between the age at which aggression first appears in the group and the age of sexual maturity in the isolated individuals ($r = 0.49$; t test, df 39, $p < 0.01$). In the juvenile females this correlation is completely absent ($r = 0.01$, df 52). Perhaps juvenile females somehow compensate for the initial retardation in development, or alternatively, the age of first aggression in the groups (with roughly equal proportions of juveniles of either sex) may be more dependent on the developmental stage of juvenile males. Whatever the cause for this discrepancy between juveniles of the two sexes may be, this is good evidence for a dependence of developmental rate of juvenile males on group-size. It should be noted too that individuals which became sexually mature at an age of 215 days or more after fertilization have been excluded from the calculations (13 individuals in C-0), either because some of these could not be sexed or because they matured much later than 215 days. In any case, the onset of aggression in the larger groups seems to be postponed.

Now I return to the hypothesis, posed above, that juveniles of the JH and JL line differ in their rate of general development. The first indication for this was the establishment of a difference in the age of onset of aggression between groups (of fixed size) of JH-3 and JL-3 juveniles (see section 4.6.1.), mentioned above. In addition, such a difference seems also plausible from Figs 14 and 15. The groups were separated from the progeny of the JH-2 and JL-2 parents at an age of 21 days after fertilization, so weeks before the onset of aggression. Further, the fry from which these groups were separated consisted of about equal numbers in the high and low line. In this case a difference in developmental rate due to the availability of food seems very unlikely. Yet this inter-line difference in the age of onset of aggression probably reflects an inter-line difference in developmental rate, since the mean age of sexual maturity also is different between the high and low line. In the third generation of selection juvenile males and females of the JL line reached sexual maturity later than their generation-mates of the high line (♂♂: JH-3 117.8 ± 3.6, JL-3 130.7 ± 5.2; ♀♀: JH-3 121.9 ± 3.8, JL-3 134.6 ± 5.6; Mann-Whitney U test, 1-tailed, $p < 0.05$ for both sexes).

In comparison to the base population, the difference in developmental rate between the JH and JL line is more likely to be side-effect of the selection for juvenile aggressiveness. This assumption is supported by the negative correlation that exists across generations between the mean JAS and the mean age of sexual maturity (Fig. 16): juvenile ♂♂: $\hat{y} = -0.185x + 127.49$, $r = -0.37$, df 5, $p > 0.10$, and juvenile ♀♀: $\hat{y} = -0.663x + 142.38$, $r = -0.76$, df 5, $p < 0.05$. However, for juvenile males the correlation coefficient does not reach significance, due to the relatively early age of sexual maturity in JL-2 males. For this no explanation can be offered. Individuals that matured at an age of 215 days or more (C-2: 1, JH-1: 7, JH-2: 0, JH-3: 0, JL-1: 1, JL-2: 2, JL-3: 6) were not included in the calculations, nor was C-0, since in the base population the developmental rate has been influenced by the group-size (see above). Contrary to C-0, juvenile females of the selection lines also show changes in developmental rate. This indicates that there is a different causation for the variation in developmental rate in the base population and in the selection lines, the former being due to external (food) causes, the latter probably due to internal causes, altered by selection for juvenile aggressiveness.

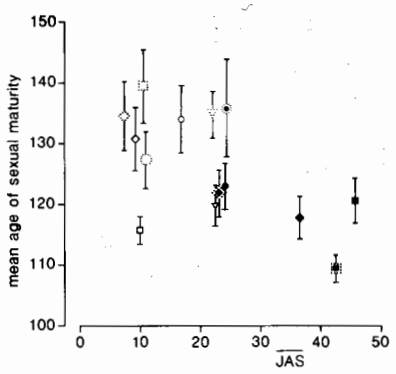

Fig. 16. Mean age of sexual maturity as a function of mean juvenile aggression score (JAS) in the juvenile aggression lines. Legends as in Fig. 14; closed contours = ♂♂, broken contours = ♀♀. Bars represent two standard errors of the means.

3.4. Territorial aggression lines.

For the territorial aggression lines the following topics are reviewed: responses to selection for territorial aggression score (TAS) and female aggression score (FAS) in section 3.4.1., estimations of the realized heritabilities for those scores in section 3.4.2., and finally changes in territorial aggresssion and female aggression with time elapsed since the onset of sexual maturity in these selection lines in section 3.4.3.

3.4.1. *Responses.*

3.4.1.1. Males.

In section 2.4.1. it was shown that males whose nests are located in the front-half of the tank tended to be more aggressive during the male tests. This tendency, however, cannot explain the difference in response of the two territorial aggression lines (see below), since the males of the low line tend to build their first nest closer to the front pane than males of the high line (Fig. 17: TH-(1+2+3) front:non-front = 16:26 and TL-(1+2+3) front:non-front = 21:11; χ^2 test, 2-tailed, $0.02 < p < 0.05$). This difference in nest-site choice is also in force for subsequent nests and consequently the opponent was offered on the average closer to the nest in the TL line than in the TH line (positions of the nests during the valid male tests: TH-(1+2+3) front:non-front = 58:118 and TL-(1+2+3) front:non-front = 75:60; χ^2 test, 2-tailed, $p < 0.001$). The possible cause for the different nest-site choice of TL and TH males will be discussed in section 4.3.3.2.

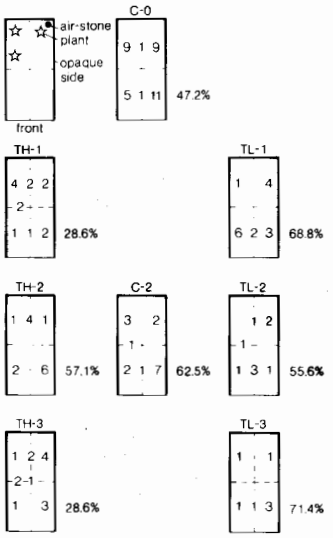

Fig. 17. Locations of the first nests (when the bottom is divided in four imaginary areas) of TH, TL and C line males, and % of first nests in the front half of tanks (excluding border area between front and back half).

In reproductive males the response to selection for increasing and decreasing territorial aggressiveness is highly asymmetric (Fig. 18: mean scores and Fig. 19: frequency distributions of scores). In the high line the response is absent: it is not significantly different from the level of C-2 (Mann-Whitney U test, 1-tailed, $p > 0.05$, for all generations of TH). Compared to the C-0 level only the first selected generation shows a significant increase ($p < 0.01$) but later generations do not differ significantly from the territorial aggression scores in C-0.

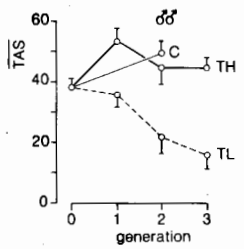

Fig. 18. Responses of males to selection for territorial aggressiveness. $\overline{TAS}$ = mean territorial aggression score. Lengths of bars represent one standard error of the mean.

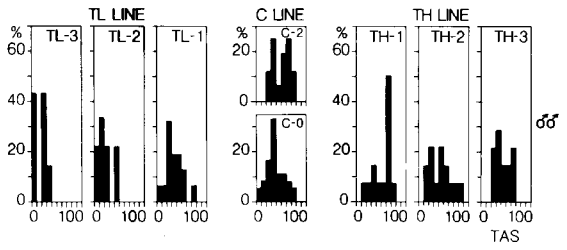

Fig. 19. Frequency distributions of territorial aggression scores (TAS) of males from the TL, C and TH line.

The difference in mean TAS between C-0 and C-2 could have resulted from a non-significant difference in nest-site between C-0 and C-2 males (Fig. 17: first nests, front:non-front = 17:19 and C-2 front:non-front = 10:6; χ^2 test, 2-tailed, $0.30 < p < 0.50$), which leads in C-2, compared with C-0, to significantly more valid male tests in which the nest is located in the front-half (nests during valid tests, C-0 front:non-front = 52:79 and C-2 front:non-front = 40:27; χ^2 test, 2-tailed, $0.01 < p < 0.02$). The different nest-site choice of C-0 and C-2 males might have arisen from the somewhat different test schedule of C-0 juveniles (see section 3.2.1.). In that respect C-2 is, in spite of the smaller sample size, more comparable to the mean scores of the selected generations. Possibly therefore the increase in TH-1 (compared to C-0) is questionable.

In the low line there is a strong response to selection. Even the first selected generation differs significantly from C-2 and TH-1 (though not from C-0) at the 5% level. In the third generation the difference is even more pronounced ($p < 0.01$ compared to C-0, C-2 and TH-3).

The failure of response in the high line cannot be attributed to methodological limitations. In the male tests the most extreme territorial aggression scores reach 70-80% biting-bumping time in the test-period (Fig. 19), while territorial aggression in the separate tests occasionally can take up even more of the total test-time.

The absence of response in the high line might have resulted from limitations in breeding, caused for example by limitations in spawning and/or selective embryonic death. An example of such limitations has been analysed for the JH line in section 3.3.1. Other selection lines, and even the control line, were not safeguarded against these difficulties in breeding, but in most cases these did not take on such serious forms that they really restricted breeding the selected parents. Although it cannot be entirely excluded that the above mentioned limitations have contributed to the lack of response in the TH line, this possibility seems unlikely, particularly since females of the TH line easily respond to selection for an increased aggressiveness (see next section). Other, more theoretical

explanations for an asymmetry of response to selection in opposite directions will be dealt with in section 4.4.

Besides the JH line, there were two more selection lines in which limitations in spawning and or embryonic death seriously handicapped breeding, viz. the DH line and the TL line. In clutches of the DH line viability of the eggs decreased with the progress of selection (see section 3.5.1.). In the TL line the male's low intensity courtship behaviour reduced the probability of spawning. This became especially evident in mating the selected TL-3 parents. The three selected males were in reproductive condition in that they possessed a nest and showed nest directed activities, though at a low level. Introduction of a ripe female in their tank often failed to evoke any sexual behaviour. In total we made 80 mating-attempts, four of which resulted in fertilized eggs. Of these four successful matings only one produced viable offspring.

It is generally accepted that the establishment of a territory precedes the nestbuilding phase in the three-spined stickleback. Several authors state that the establishment of a territory is restricted to the breeding cycle: "....the three-spined stickleback where territories are exclusively connected with breeding activities." (VAN DEN ASSEM, 1967), and "....the nestbuilding phase is marked by the onset of territorial behaviour,...." (WOOTTON, 1976). In the present study observations made on juvenile sticklebacks (groups of full sibs, see section 4.2.2.) clearly showed that in some groups areas were defended months before nestbuilding started. In the most extreme cases this phenomenon was manifested in the 8th week after fertilization: tiny juvenile sticklebacks attacked one another at the boundaries of imaginary areas and repeatedly returned to the centre of these areas. Some of them obviously failed to gain a territory and were continuously chased away to another area. The territories in the juvenile phase are, however, probably rather temporary, and not restricted to the bottom of the tank.

3.4.1.2. Females.

The degree of ripeness during the female tests might have interfered with selection for female aggressiveness, since in the base population ripe females were less aggressive in female tests than non-ripe ones, although not significantly (see section 2.3.). The proportion of ripe females during the valid female tests is, however, not different between the high and low line (Table 18; all differences between TH and TL not significant, χ^2 test, 2-tailed) and so this factor cannot contribute to the difference in female aggressiveness in the two territorial aggression lines (see below). I cannot suggest an explanation for the low incidence of ripe C-0 females during the tests (see Table 18).

TABLE 18. Incidence of ripe females during the valid female tests in the TH, TL and C line

Generation	Incidence of ripe ♀♀ in valid female tests					
	TH		TL		C	
	n	%	n	%	n	%
0					37	23.6
1	19	45.2	25	52.1		
2	20	50.0	10	43.5	33	40.7
3	26	52.0	15	42.9		
Total	65	49.2	50	47.2	70	29.4

In contrast to the males, the responses to selection for female aggression in opposite directions are almost symmetrical (Fig. 20). The increase of mean FAS in the TH line is already significant in the second generation of selection (Mann-Whitney U-test, 1-tailed, $p < 0.05$, compared to

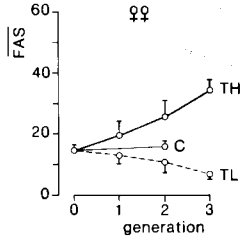

Fig. 20. Responses of females to selection for female aggressiveness. $\overline{FAS}$ = mean female aggression score. Lengths of bars represent one standard error of the mean.

C-0 and C-2), as is the difference between the high and low line ($p < 0.05$). In the third generation the increase has become more pronounced ($p < 0.01$, compared to C-0 and C-2), just like the decrease of mean score in the TL line ($p < 0.05$, compared to C-0 and $p < 0.01$, compared to C-2). Thus female aggressiveness is as easily enhanced as it is lowered (see also the frequency distributions of FAS in Fig. 21).

In female tests, the mean reponsiveness (expressed as the % valid tests) varies considerably among females, and for a given female responsiveness is not constant in repeated tests either. Responsiveness is obviously related to two variables, viz. aggressiveness and degree of ripeness. As in males (see section 4.3.3.2.), mean responsiveness and mean FAS are positively correlated across the generation-means (Fig. 22; with C line: $r = 0.59$, t-test, 2-tailed, $0.10 < p < 0.20$; without C line: $r = 0.93$, $0.001 < p < 0.01$). The reason for the high responsiveness of C line females is unclear. In

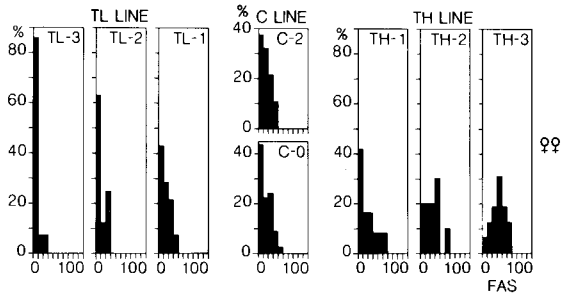

Fig. 21. Frequency distributions of female aggression scores (FAS) of females from the TL, C and TH line.

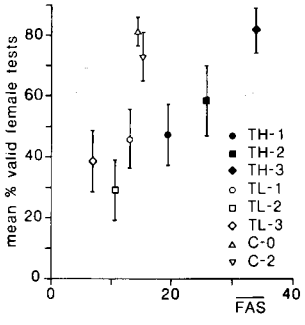

Fig. 22. The correlation of the mean responsiveness during female tests (expressed as mean % valid tests) with the mean female aggression score ($\overline{FAS}$) in the TH, TL and C line. Lengths of bars represent two standard errors of the mean.

C-0 (but not in C-2) it can probably be traced to the higher frequency of juvenile tests, compared to the generations after C-0 (see section 3.2.1.). The responsiveness during the juvenile tests was even more affected by this change in test frequency (see section 4.3.1.).

The influence of the other variable, viz. the degree of ripeness, on the responsiveness in female tests, appears from the lower incidence of ripeness in the invalid tests (Table 19), compared to the valid tests (Table 18), especially in the TL line (valid *vs* invalid, χ^2 test, 2-tailed, TH: $0.20 < p < 0.30$ and TL: $0.001 < p < 0.01$). Ripe TL females obviously respond better in the female test than do non-ripe ones. In itself this is not astounding, since in tanks with only females (the usual way of providing regularly ripe females in our laboratory) one can often see ripe females in head-up posture follow other females. This enhanced responsiveness of ripe females to the test stimulus is, however, less clear in TH females, possibly because responsiveness of non-ripe females is already much higher in the TH line than in the TL line (non-ripe TH females, valid:invalid = 67:75 and non-ripe TL females, 56:187; χ^2 test, 2-tailed, $p < 0.001$).

The weekly scored incidence of ripeness of TH and TL females can be used to analyse the possibility of a differnce in overall incidence of ripeness between high and low line females, that is a differnce in length of interspawning interval. We cannot get a complete

TABLE 19. Incidence of ripe females during the invalid female tests in the TH, TL and C line

Generation	Incidence of ripe ♀♀ in invalid female tests					
	TH		TL		C	
	n	%	n	%	n	%
0					7	24.1
1	28	35.0	27	27.6		
2	22	57.9	28	25.7	13	37.1
3	4	36.4	26	42.6		
Total	54	41.9	81	30.2	20	31.3

picture of the frequency of spawning from these data, since the degree of ripeness was judged but once a week, and under our experimental conditions females may spawn every three or four days with the ripe state lasting half a day or so. As a consequence of the difference in responsiveness between females of the high and low line (see above), TL females had to be tested over a longer period than TH females in order to obtain four valid female tests per female. It is a reasonable assumption that at later ages the incidence of ripeness is reduced (BAGGERMAN, 1957), so that for an appropriate comparison the incidence of ripeness of TH and TL females should be considered during identical time periods. When this is done for the first four female tests, whether valid or invalid (Table 20) no difference in the incidence of ripeness between the two lines is found (valid + invalid tests; TH ripe:non-ripe = 79:82 and TL 83:121; χ^2 test, 2-tailed, $0.10 < p < 0.20$). The conclusion must be that females of the TH and TL line do not differ significantly with respect to the incidence of ripeness during about the first month after the onset of the breeding period, although a tendency towards a lower incidence of ripeness in TL females is present. Comparison of Tables 18, 19 and 20 shows that at later ages the incidence of ripeness is reduced indeed, especially in the TL line (TH: first four tests, ripe:non-ripe = 79:82 and later tests, 40:60; χ^2 test, 1-tailed, $0.05 < p < 0.10$ and TL: first four tests, ripe:non-ripe = 83:121 and later tests, 48:122; χ^2 test, 1-tailed, $0.005 < p < 0.01$).

TABLE 20. Incidence of ripe females during the first four female tests, separated according to valid and invalid tests, in the TH, TL and C line

Generation	Incidence of ripe ♀♀ in first 4 female tests											
	TH				TL				C			
	Valid		Invalid		Valid		Invalid		Valid		Invalid	
	n	%	n	%	n	%	n	%	n	%	n	%
0									32	21.8	6	26.1
1	13	59.1	9	39.1	13	46.4	12	42.9				
2	14	45.2	15	53.6	9	47.4	17	33.3	26	40.0	8	34.8
3	24	51.1	4	40.0	13	44.8	19	40.4				
Total	51	51.0	28	45.9	35	46.1	48	37.5	58	27.4	14	30.4

3.4.2. *Heritabilities.*

In the previous two sections the selection experiments for territorial aggressiveness in reproductive males and females were considered with respect to selection success and factors which could interfere with such selection, viz. nest location and ripeness. In this section the selection process is analysed in a more quantitative way in order to arrive at estimates of realized heritabilities for territorial aggressiveness and female aggressiveness. Quantitative data on the two-way selection for territorial aggressiveness and female aggressiveness are presented in Table 21.

TABLE 21. Results of selection for increased (TH line) and decreased (TL line) territorial aggressiveness (♂♂ and ♀♀)

	TH Selection Line						C Line	
Genera-	Tested ♂♂		Selected ♂♂		Cumulative	Cumulative	Tested ♂♂	
tion	n	$\overline{TAS}$	n	$\overline{TAS}$	R	weighted S	n	$\overline{TAS}$
	36	38.1	3	66.3	0.0	0.0	36	38.1
	14	53.2	2	66.2	+ 9.5	+28.6	—	—
	14	44.7	3	73.0	+ 1.0	+42.3	16	49.3
	14	44.5	2	61.0	+ 0.8	+70.7	—	—
	Tested ♀♀		Selected ♀♀				Tested ♀♀	
	n	$\overline{FAS}$	n	$\overline{FAS}$			n	$\overline{FAS}$
	46	14.5	2	26.8	0.0	0.0	46	14.5
	12	19.4	3	41.6	+ 4.5	+12.3	—	—
	10	25.8	3	43.3	+10.9	+34.7	19	15.3
	16	34.2	2	46.3	+19.3	+58.6	—	—

	TL Selection Line						C Line	
Genera-	Tested ♂♂		Selected ♂♂		Cumulative	Cumulative	Tested ♂♂	
tion	n	$\overline{TAS}$	n	$\overline{TAS}$	R	weighted S	n	$\overline{TAS}$
	36	38.1	4	26.0	0.0	0.0	36	38.1
	16	35.9	2	11.1	- 7.8	-11.8	—	—
2	9	21.5	2	9.6	-22.2	-36.0	16	49.3
3	7	15.8	1	0.8	-27.9	-48.5	—	—
	Tested ♀♀		Selected ♀♀				Tested ♀♀	
	n	$\overline{FAS}$	n	$\overline{FAS}$			n	$\overline{FAS}$
0	46	14.5	4	3.9	0.0	0.0	46	14.5
1	14	13.1	2	5.1	-1.8	-10.2	—	—
2	8	10.7	2	0.7	-4.2	-18.3	19	15.3
3	14	6.9	1	0.9	-8.0	-27.9	—	—

$\overline{TAS}$ = mean territorial aggression score, $\overline{FAS}$ = mean female aggression score, R = response in regard to mean of C-0 and C-2, S = selection differential, C = control line, n = number of fish.

The numbers of fish tested (see Table 21) vary somewhat across generations, especially in the TL line, for two reasons. First, the sex ratio of the isolated TL fish became progressively more skewed (♂♂:♀♀, TL-1 17:17, TL-2 9:18, TL-3 7:23; χ^2 test, 2-tailed, TL-2 $0.05 < p < 0.10$, TL-3 $0.001 < p < 0.01$). Since this feature was not observed in the other selection lines, it is unlikely that biased sampling is involved. This skewed sex ratio in the TL line might result from so-called "atypical sex determination", which is not an uncommon natural phenomenon in teleost fish (*e.g.* KALLMAN, 1975; CHAN & YEUNG, 1983). It implies that embryos with an originally female genotype develop into functional males and *vice versa.* Hence, selection for a decreased territorial aggressiveness may have influenced the process of sex differentiation in TL juveniles with a male genotype. In the next chapter (chapter 4) it is shown that the level of androgens is very likely reduced in TL males. Since sex steroids are known to have a potent effect on the process of sex differentiation in fishes (*e.g.* YAMAMOTO, 1969), the level of androgens (or the level of gonadotropins) may have been too low in part of the TL male genotypes to induce a male phenotype. There is some evidence to suggest that atypical sex determination occurs, though rarely, in the three-spined stickleback; in the literature there is only one report which could point to it, viz. the higher percentages of females found by LINDSEY (1962) under crowded rearing conditions and also from eggs reared at higher temperature. Further, there are indications that consistent removal of males from a group of sticklebacks leads to "sex reversal" of females (SEVENSTER, pers. comm.).

In groups of TL fish, sampled from the same fry as the isolated fish, the sex ratio was much less skew (♂♂:non-♂♂ (*i.e.*, adult fish without any sign of red in the mouth cavity), TL-1 21:19, TL-2 5:9, TL-3 14:19; χ^2 test, 2-tailed, $p > 0.70$, $p > 0.20$, $p > 0.30$, respectively). Furthermore, no deviation from a sex ratio of one was found in a complete TL-4 clutch, kept as a group (♂♂:♀♀ = 24:26). Possibly then, there is a mutual influence of group members on sex development.

It is interesting to note, that selection for high dominance ability in the paradise fish (*Macropodus opercularis*), which in itself was unsuccessful, caused a pronounced male bias in the sex ratios in the high line (FRANCIS, 1984).

A second reason for the variable number of fish tested in the TL line is the diminished responsiveness of TL females during the female tests, as shown in the previous section. Some of these females refused to respond (proportion of non-responders, TL-1: 3/17, TL-2: 10/18, TL-3: 7/21), in spite of the large number of test trials to which they were subjected (in some cases exceeding 10). These females were rejected in the selection procedure to allow for a quantitative approach of the selection experiments for overt aggression. In the reproductive males such non-responders were quite rare.

The difference in selection differential between the high and low line and between the sexes can be ascribed to scale-effects, as argued for the juvenile aggression lines in section 3.3.2. With the figures presented in Table 21 realized heritabilities are calculated from the regression of cumulative response against cumulative weighted selection differential (Table 22). The realized h^2 for TAS in the TL line is rather high even if compared with the value for the repeatability of territorial aggression and for the doubled full sib correlation of TAS in C-0 (cf. Tables 12, 14 and 22). As argued for JAS in section 3.3.2., this probably is due to the asymmetry of response to selection in opposite directions, which is most striking for TAS. The relative amount of additive genetic variance for TAS in C-0 is therefore probably best approximated by the realized h^2

computed from the difference in response of the high and low line (see Table 22). In females the response to selection for FAS in opposite directions is nearly symmetrical. The realized h^2 in either direction is therefore presumed to correspond well with the relative amount of additive genetic variance of female aggressiveness in the base population.

TABLE 22. Realized heritabilities (h^2) for TAS and FAS in the TH and TL line, as estimated from the regression of the selection response on the cumulative selection differential

Type of selection	h^2	s_b	r	p
TH ♂♂	-0.01	0.11	-0.07	$0.25 < p < 0.35$
TL ♂♂	0.58	0.02	1.00	$0.0005 < p < 0.001$
TH + TL ♂♂	0.23	0.05	0.96	$0.01 < p < 0.025$
TH ♀♀	0.32	0.01	1.00	$0.0005 < p < 0.001$
TL ♀♀	0.29	0.04	0.98	$0.005 < p < 0.01$
TH + TL ♀♀	0.31	0.01	1.00	$0.001 < p < 0.005$

s_b = standard deviation of the regression coefficient, r = correlation coefficient, p = probability.

In the above treatment of the selection results both parents were assumed to have an equal share in the territorial aggression scores of their sons and the female aggression scores of their daughters, so that the slopes of the calculated regression lines can be regarded as estimates of the realized heritabilities. To check the validity of this assumption, cross-regression lines are calculated, in the same way as for selection for JAS (section 3.3.2.), according to the method given in section 3.2.4. The slopes of the resulting cross-regression lines (Table 23) cannot be compared directly to the realized heritabilities in Table 22 since the mean scores of males and females differ considerably. The cross-correlation coefficients are nevertheless almost as high as the correlation coefficients computed within the sexes (see Table 22). I therefore conclude that both parents contribute roughly equally to the aggression scores of their sons and daughters in the sexual phase.

TABLE 23. Cross-regression for TAS and FAS in the TH and TL line

Type of regression	b_{RS}	s_b	r	p
R(TL ♂♂) - S(TL ♀♀)	1.07	0.16	0.98	$0.01 < p < 0.025$
R(TH ♀♀) - S(TH ♂♂)	0.30	0.04	0.99	$0.005 < p < 0.01$
R(TL ♀♀) - S(TL ♂♂)	0.15	0.03	0.97	$0.01 < p < 0.025$

For explanation see text. R = response, S = selection differential, b_{RS} = regression coefficient of R on S. For remaining symbols see previous table.

3.4.3. *Changes in aggression as a function of age.*

Although individuals in the territorial aggression lines were selected with respect to the mean relative duration of overt aggression during the aggression tests, it is interesting to view the outcomes of the separate tests as a function of time elapsed since the onset of the breeding period. In males this onset is defined by the completion of the first nest. For males of the base population it was already shown that territorial aggressiveness remains fairly constant with age, especially during the first month after the onset of the sexual phase (see section 2.4.1.). This also applies to territorial aggressiveness in C-2 and in the three generations of the TH line (Fig. 23). After the first four male tests the mean aggression level is somewhat more variable, probably because of the reduced number of males on which those means are based. The lack of response in males of the high line is obvious, if compared to the C-2 level; only territorial aggression is slightly enhanced in TH-1. The level of aggression in the high line is somewhat higher than that for C-0, although this certainly does not become more pronounced as selection procedes. As argued in section 3.4.1.1., the C-0 level has probably been lowered, partly because a larger proportion of C-0 than C-2 males preferred to build their nest in the back-half of the tank. Compared to C-2 (not C-0), the TL line's territorial aggressiveness has already decreased in the first selected generation, apart from the high level in the first week after nest-building. In

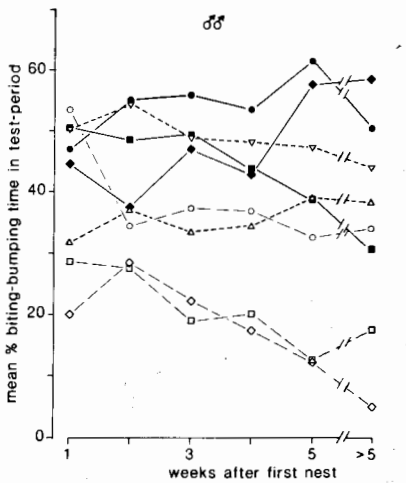

Fig. 23. Mean territorial aggressiveness of the TH, TL and C line males as a function of time elapsed since the completion of the first nest. Symbols as in Fig. 22.

TL-2 and TL-3 the decrease continues; not only is the initial level lowered, but there is also a clear tendency of territorial aggressiveness to decrease with age, which may be partly a side-effect of the reduced responsiveness of TL males (see section 4.3.3.2.).

The mean aggression level of females shows generally larger fluctuations than for males during the breeding period, the onset of which is marked by the attainment of first ripeness (Fig. 24). These fluctuations are due to the combined effect of a lower mean responsiveness of females in the aggression tests and a variable frequency of ripeness within and between females during the tests (see section 3.4.1.2.). Analysis of mean female aggressiveness as a function of time elapsed since the onset of the breeding period revealed no clear trends. The high aggression line shows increased aggression throughout the breeding period, as opposed to the decreased level observed in the low aggression line, a difference that is most pronounced in the third generation of the territorial aggression lines.

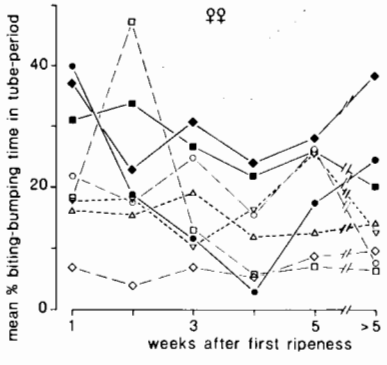

Fig. 24. Mean female aggressiveness of the TH, TL and C line females as a function of time elapsed since first becoming gravid. Symbols as in Fig. 22.

3.5. Dominance lines.

Dominance ability is defined as the rank of a reproductive, isolated male in the linear order of dominance that exists between a number of isolated males (see section 2.4.3.). Selection for dominance ability poses specific problems. Since successive generations cannot be compared directly on the basis of their mean dominance ability, the response to selection must be assessed in a way different from that used in the other selection lines (see section 3.5.1.). The difficulty in estimating the realized heritability

of dominance ability, viz. the discontinuous variation of dominance as it is defined in the present study, is discussed in section 3.5.2.

In a previous publication on dominance in the stickleback (BAKKER & SEVENSTER, 1983) it was shown that colouration and experience are important determinants of dominance in male three-spined sticklebacks. Differences in aggressiveness (as assessed in male tests) seem to play only a very minor role in the outcome of a dominance test. As an extension of that work some more data concerning the colouration of the males of the dominance lines in the course of the selection experiment are presented in section 3.5.3. Furthermore, the relation of dominance and size of the males is discussed.

3.5.1. *Responses.*

The males of the dominance lines were submitted every generation to dominance tests after the assessment of their territorial aggression scores. Within lines all possible combinations of two males were tested. It then appears that the isolated males tested in this way fit into a linear order of dominance (see also BAKKER & SEVENSTER, 1983).

Some test results deviate from linearity (''wrong'' tests) and others have to be scored as undecided if aggression or flight is absent within at least 15 min after introduction of the two males in a tank which is unfamiliar to both. About half of these ''wrong'' tests can be ascribed to ''wrong'' inferiority as the result of experience of inferiority in the preceding dominance test against one of the three most dominant males in the group (see BAKKER & SEVENSTER, 1983). The comparatively frequent occurrence of ''wrong'' tests in the DH line and of undecided tests in the low line (Table 24) is probably due to reduced variation of dominance ability during selection of extreme phenotypes. This reduction cannot be deduced from the linear orders of dominance, for the ranks of the males are determined only from the number of tests won or lost and are therefore equidistant. This does not mean, however, that dominance relationships are etablished with equal effort between males at adjacent ranks in the order of dominance. Reduction of variation in dominance ability in the lower direction (DL line) is likely to result in a larger number of undecided tests. With a reduction of variation in dominance ability in the higher direction (DH line) prolonged fights are to be expected. Indeed, BAKKER & SEVENSTER (1983) found that roundabout fighting is seen more frequently in the DH line than in the DL line, and they showed that in tests with roundabout fights a decision is delayed, in the DH line even more than in the DL line. Nevertheless,

TABLE 24. Number of fish used in the dominance lines, incidence of "wrong" tests (test results deviating from linearity) and incidence of undecided tests (no aggression or flight within 15 min after introduction)

Line	Genera-tion	Number tested	Number of tests	♂♂ "Wrong" tests n	%	Undecided tests n	%	Number selected	♀♀[a] Number selected
DH	0	8[b]	28	1	3.6	0	0.0	4	6
	1	12	66	7	10.6	0	0.0	4	4
	2	10	45	4	8.9	0	0.0	2	2
	3	10	45	3	6.7	2[c]	4.4	2	2
DL	0	8[b]	28	1	3.6	0	0.0	4	4
	1	14	91	3	3.3	2	2.2	7	5
	2	16	120	5	4.2	16	13.3	4	4
	3	10	45	0	0.0	5	11.1	2	2

[a] = ♀ ♀ selected according to the rank of their brothers, [b] = most dominant and most inferior males separated from the base population (see section 2.1.2.5.), [c] = males still roundabout fighting after 45 min.

both lines show an increase during selection in the incidence of roundabout fighting and in the time it takes before a decision is made in first dominance tests that include roundabout fighting (Table 25). This again points to a decreased variation of dominance ability in both lines. Finally, the relatively higher incidence of "wrong" tests in the high line probably reflects the more severe impact of an inferiority experience in the DH line, since fighting can be so vigorous between DH males.

TABLE 25. Incidence of dominance tests with roundabout fighting (+R) and mean duration of decision (in sec) in first tests with roundabout fighting for both dominance lines

Line	Generation	Incidence of +R tests n	%	Mean duration of decision in first +R tests
DH	1	20	30.3	510
	2	19	42.2	600
	3	21	46.7	1500
DL	1	12	13.2	372
	2	21	17.5	420
	3	13	28.9	562

In both lines some of the males with the most extreme ranks (for numbers see Table 24) were selected each generation to father the next generation. To determine the joint response to two-way selection for dominance, in each generation inter-line dominance tests were held with males randomly chosen from both lines. The deviation from random (50% gain by one of the lines) is a measure of the difference in mean dominance ability between the DH and DL line. Table 26 (middle columns) shows that in the course of selection the discrepancy between the lines increases, and in generation 3 reaches the 1% level of significance (χ^2-test, 1-tailed). So selection for dominance ability has been successful in one or both directions. From confrontations of high and low line males with those of the control line (gen. 2) it is most likely that the increasing difference in mean dominance ability between the selection lines is due to a decreasing mean dominance ability of the DL line. The high line probably does not respond to selection for increased dominance ability (Table 26, lower middle columns). This conclusion of the lack of response in the DH line is further supported by the results of dominance tests between both dominance lines and the other selection lines (see section 4.3.3.4.).

Another way of assessing the joint response of the high and low line is to construct a joint linear order of dominance from the inter-line test results. Because of limited samples used in this assessment there are a number of alternatives from which to choose. Of these alternatives the

TABLE 26. Differences between mean dominance abilities of DH, DL and C line

Dominance tests between	Number of tests	Won by DH ♂♂ n	Won by DH ♂♂ %	Degree of non-overlap DH favoured	Degree of non-overlap DL favoured
DH-1 / DL-1	19	12	63.2^{ns}	0.24^{*}	0.18^{ns}
DH-2 / DL-2	9	6	66.7^{ns}	0.33^{**}	0.03^{ns}
DH-3 / DL-3	24	19	79.2^{**}	0.39^{**}	0.24^{*}
		Won by C ♂♂ n	Won by C ♂♂ %		
DH-2 / C-2	10	5	50.0^{ns}		
DL-2 / C-2	10	7	70.0^{ns}		

For explanation see text. Middle columns: deviations from random tested with χ^2 test (or binomial test), 1-tailed, ns = $p>0.05$, ** = $p<0.01$. Right columns: ranks of DH ♂♂ compared with ranks of DL ♂♂ in a combined order of dominance; Mann-Whitney U test, 1-tailed, ns = $p>0.05$, * = $p<0.05$, ** = $p<0.01$.

two most extreme linear orders are constructed, which I will call the "DH favoured" and the "DL favoured" order. From each of these two extreme orders the degree of non-overlap (NO, see section 3.2.2.) between the two selection lines can be calculated; thus, we get a DH favoured NO and a DL favoured NO. The real value of NO may be taken to lie midway between these two extremes. Because both lines were founded from the same base population, NO is initially minimal, that is 0. With the progress of selection NO increases (Table 26, right columns). After three generations of selection the maximum value of NO, viz. 0.5, is not yet reached, so the ranks of the males of the two dominance lines still overlap each other in the joint linear order of dominance. The low NO in the DL favoured order of generation 2 is due to the small number of inter-line dominance tests, especially between males with a low dominance ability. This causes severe overestimation of the ranks of the DL males with a low dominance ability in the DL favoured joint order of dominance.

A final item that is discussed in this section is whether the lack of response in the DH line may be explained by restrictions at breeding, as was shown for the JH line (see section 3.3.1.). Indeed, there are some differences in reproductive behaviour between DH and DL males, but under laboratory conditions they do not interfere severely with courtship and spawning. Males of the DH line were *e.g.* more apt to perform dorsal pricking (a behaviour that may occur when the male is relatively aggressive during courtship; WILZ, 1970. It tends to prolong the courtship sequence). Further, nests of DH males were small and more than once destroyed by females attempting to spawn. On the other hand, males of the DL line more frequently showed courtship activities of low intensity. Females of both lines behaved aggressively towards their males about equally often. Aggressive behaviour of females was observed either when the male was showing the nest entrance or else as a reaction to aggressive behaviour of the male. In the latter case this sometimes resulted in roundabout fighting. Extreme aggressiveness to the extent that reproduction failed, as in the JH line (see section 3.3.1.), was not observed in the dominance lines.

Aggression of females during courtship and spawning seems to some extent a result of isolation, since it was most frequently observed in the first few encounters with a male. Moreover, females grown up in groups never behaved aggressively on such occasions. The enhanced aggressiveness of isolated three-spined sticklebacks was also found for males (CULLEN's data, 1960, re-analysed using principal components analysis by HUNTINGFORD, 1976d).

Although mean number of eggs in clutches produced by selected parents do not differ between the DH and DL line and are comparable with those of the other selection lines, there is a clear difference in the development of eggs between the two lines (Table 27). In clutches of the DH line viability of the eggs is reduced with the progress of selection, while in the DL line the reverse is the case. Exact data on the viability of eggs produced by C-3 parents are lacking, but considering the number of young that hatched viability is roughly comparable to the C-2 data. Though unfortunately viability of eggs in clutches of the C line was sub-optimal, it was certainly not so low as in later generations of the DH line, when hardly a normal developing clutch could be obtained. This must be due to other causes. In section 4.4. it will be argued that the increased embryonic mortality in the DH line might be traced to a reduced viability of genotypes for "enhanced dominance".

TABLE 27. Development of eggs in clutches produced by selected parents of the DH, DL and C line

Line	Generation	Total number of clutches	Number of clutches with the following % % of well-developed eggs:			
			<5%	5-40%	40-75%	>75%
DH	1	6	-	-	1	5
	2	6	4	-	1	1
	3	12	7	5	-	-
DL	1	9	1	2	1	5
	2	4	-	1	-	3
	3	2	-	-	-	2
C	1	9	-	-	4	5
	2	9	-	2	1	6

Eggs were judged on their development 5-7 days after fertilization. Poorly developed eggs included non-fertilized, retarded and dead eggs.

3.5.2. *Heritabilities.*

The estimation of the heritability for dominance ability is a difficult matter because in this case the normality assumption is unrealistic. No non-parametric counterpart for h^2 is available, so one has to be satisfied with a h^2-estimate which is probably biased.

The joint response of both dominance lines was deduced from the results of inter-line tests and expressed as the degree of non-overlap (see Table 26). The applied selection differentials in either direction can be

expressed in an analogous manner; the ranks of the selected males within their group are related to the mean rank of their group and corrected for the number of males involved (Table 28). The most extreme values of S thus calculated are + or -0.5 (if the number of males involved is large). It will be clear that comparison of S with the response is only valid as long as the joint response is not maximal, for if all ranks of the DH males in a joint order of dominance are higher than those of DL males (*i.e.* there is no overlap), further response to selection for dominance ability has to be assessed in another way. In the present study this level is not reached.

TABLE 28. Applied selection differentials (S) in the dominance lines

Generation	Line	Mean dominance rank tested ♂♂	Mean dominance rank selected ♂♂	S	Cumulative total S
0[a]	DH	7.5	12.5	0.36	0.00
	DL	7.5	2.5	-0.36	
1	DH	6.5	10.5	0.33	0.73
	DL	7.5	4.8	-0.19	
2	DH	5.5	8.5	0.30	1.24
	DL	8.5	3.8	-0.29	
3	DH	5.5	9.5	0.40	1.83
	DL	5.5	2.5	-0.39	

S = (mean rank of selected ♂♂ - mean rank of tested ♂♂) / (number of tested ♂♂), [a] = S calculated from 14 ♂♂ of the base population with incontestable dominance relationships (see text).

The S in C-0 cannot be assessed with certainty, for C-0 males were split into four groups of equal size and the most and the least dominant male of each group determined. The selected eight males were arranged in a linear order of dominance. In theory the four most dominant males in this order of dominance could represent the four most dominant males of the whole base population (n = 4 × 8 = 32 males) and the four most inferior males the four least dominant ones of C-0. This seems an unlikely supposition. The two extreme males in one of the four groups from C-0 occupied two adjacent ranks in the linear order of dominance of the eight extreme males, so the other six members of that group can easily be ranked in the total order of dominance. The selection differential in the base population is related to the order of dominance of these 8 + 6 = 14 males.

Regression of the joint response of the DH and DL line (see Table 26, right columns) on cumulative total S (see Table 28) yields regression

coefficients of 0.21 (DH favoured) and 0.13 (DL favoured; leaving out gen. 2, see section 3.5.1.). The average slope (0.17) multiplied by 2 (because females were not selected for dominance ability), *i.e.* 0.34, is probably the best approximation of the realized h^2 for dominance.

Because of the difficulties with the underlying assumptions in the calculation of the realized h^2, I verified the method by applying it to the territorial aggression lines. Territorial aggression scores and female aggression scores can be readily transformed into ranks. With the aid of these ranks realized heritabilities for territorial and female aggressiveness can be calculated in a way comparable to that applied for the dominance lines (Table 29). The realized heritabilities calculated in this way do not deviate much from the values calculated on the basis of the more appropriate aggression scores (compare with Table 22, ♂♂:0.23 and ♀♀: 0.31). I conclude that the value of 0.34 is a reliable estimate for the realized h^2 for dominance ability.

TABLE 29. Realized heritabilities for TAS and FAS in the TH and TL line when aggression scores are expressed as ranks

Generation	♂♂ (TH and TL) R(expressed as NO)	cum. total S	♀♀ (TH and TL) R(expressed as NO)	cum. total S
0	0.00	0.00	0.00	0.00
1	0.25	0.66	0.12	0.62
2	0.33	1.35	0.30	1.17
3	0.47	2.02	0.48	1.90
b_{RS}	0.22		0.26	

For explanation see text. R = response, S = selection differential, NO = degree of non-overlap, b_{RS} = regression coefficient of R on S.

3.5.3. *Dominance hierarchy, length and colouration.*

The concept of the linear dominance hierarchy was developed by SCHJELDERUP-EBBE (1922) for chickens. In quite a few fish species a nip-order, comparable to such a peck-order in chickens, can be observed in groups of limited size. These nip-dominance hierarchies, sometimes called nip-right hierarchies depending on the relative frequency of, among other things, "reverse nipping" (see MYRBERG, 1972), were described *e.g.* for young kamloops trout (*Salmo gairdneri* kamloops) (STRINGER & HOAR, 1955), and other trout species (*Salmo trutta*, *Salmo gairdneri*) (JENKINS, 1969), for the cyprinids *Danio malabaricus* (NOBLE, 1938; HAAS, 1956), *Barbus pentazona* (NOBLE, 1938), for the poeciliid fish *Poecilia sphenops* (PARZEFALL, 1969, 1974), *Poecilia maculatus* (BRADDOCK, 1945), *Poecilia helleri* (NOBLE & BORNE, 1938), the guppy *Poecilia reticulata* (GORLICK, 1976), *Gambusia hurtadoi* MCALISTER, 1958), for the bicolor

damselfish *Eupomacentrus partitus* (MYRBERG, 1972), and for the Midas cichlid *Cichlasoma citrinellum* (BARLOW & BALLIN, 1976; BARLOW & WALLACH, 1976). Under certain circumstances and in certain periods of their lives the social organization of these, and surely many more, species can be characterized by a nip-dominance hierarchy. In some species, *e.g.* in the brown and rainbow trout and in the bicolor damselfish, the hierarchy coincides with some territorial structure. These dominance hierarchies are in general restricted to groups of limited size, for individual recognition is a prerequisite for the establishment of such more or less stable hierarchies.

Though stable and linear dominance hierarchies are not known in the three-spined stickleback, there are a few studies in which, under certain conditions, the presence of some kind of dominance hierarchy is indicated. One sort of nip-dominance hierarchy between rival males was described by VAN DEN ASSEM (1967). Another remarkable dominance hierarchy in the three-spined stickleback was demonstrated by LI & OWINGS (1978a). In small groups of females dominance relationships were established, the dominant one maintaining larger home ranges and engaging in more aggressive encounters. Furthermore, during courtship dominant females had priority of access to males.

The linear order of dominance, as assessed in the present study within a group of isolated males, should be distinguished from the hierarchies just mentioned. The males were kept in isolation and individual recognition was ruled out because all confrontations were unique. The rank of a male in such a linear order of dominance was determined purely by the number of dominance tests he won or lost. Probably such a linear order of dominance corresponds to some extent to the hierarchy in a rival situation, described by VAN DEN ASSEM (1967). In domestic chickens at least, the outcomes of initial pair contests and peck-order ranks of the same individuals are moderately to highly correlated (COLLIAS, 1943; GUHL, 1962).

3.5.3.1. Length.

In a number of fish species there is a distinct correlation between the rank in a nip-dominance hierarchy and the size of the fish, like *e.g.* in the bicolor damselfish *Eupomacentrus partitus* (MYRBERG, 1972), the toothcarp *Poecilia sphenops* (PARZEFALL, 1969, 1974), *Poecilia maculatus* (BRADDOCK, 1945) and the guppy *Poecilia reticulata* (GORLICK, 1976). In the three-spined stickleback size is not an important variable for a male's rank in a linear order of dominance, at least not within the range of body lengths

of fish in the present study (Table 30). If the age at which length was assessed is taken into account, mean lengths of males belonging to the DH, DL or C line do not differ. Within the linear orders of dominance a correlation between rank and length is lacking, with the exception of the order of dominance of DL-2 males. However, the correlation here is negative, that is smaller males tend to have higher ranks, and therefore is the reverse of that expected on the basis of data from the literature. It is of course possible that this correlation is merely spurious. Anyhow, size does not seem to play a noticeable part in the determination of dominance in this study.

TABLE 30. Relation between dominance ability and standard length

Line	Generation	Number of males	Mean length ± S.D.[a]	Correlation[b] rank - length
C	0	8	5.6 ± 0.3	-0.16^{ns}
	2	16	5.0 ± 0.3	—
DH	1	12	4.9 ± 0.3	$+0.27^{ns}$
	2	10	4.9 ± 0.2	$+0.08^{ns}$
	3	9	4.6 ± 0.2	-0.09^{ns}
DL	1	14	5.0 ± 0.4	-0.39^{ns}
	2	16	4.9 ± 0.4	-0.52^{*}
	3	10	4.7 ± 0.4	$+0.07^{ns}$

[a] = Age at which standard length was measured: C-0 15 months, DH-1 and DL-1 9-10 months, DH-2 and DL-2 8-9 months, C-2 9-10 months, DH-3 and DL-3 9-10 months.
[b] = Spearman rank correlation coefficient, 2-tailed, ns = $p > 0.10$, * = $0.02 < p < 0.05$.

It is also possible that the discrepancy in time between rank assessment and length determination caused the negative correlation between rank and length in DL-2 males. The lower ranking males of DL-2 may have had a shorter breeding period. Termination of the breeding period is characterized by a fall in androgen level (*e.g.* BORG, 1981). This might facilitate further growth of the males, since androgens might suppress growth, as is the case in poeciliid fish (see *e.g.* KALLMAN, 1975).

3.5.3.2. Colouration.

Another variable, which in several fish species is correlated with a male's position in dominance hierarchy, is colouration. Striking examples are the correlations of colouration with social rank in *Gambusia hurtadoi* (MCALISTER, 1958) and in *Cichlasoma citrinellum* (BARLOW & BALLIN, 1976; BARLOW & WALLACH, 1976). In the three-spined stickleback male brightness of colouration is an important determinant of dominance (BAKKER & SEVENSTER, 1983). It is therefore not surprising to find that

selection for dominance is accompanied by a change in colouration: in the DL line dull males are more numerous than in the DH line (see BAKKER & SEVENSTER, 1983). The colouration was assessed just after the dominance tests by stimulation with a ripe female, but such data were only available for the second and third selected generations.

A more complete picture of changes in brightness of males in both selection lines, as compared to the colouration of C males, can be gained from other colour assessments made just after each male test and courtship test. The colouration, as laid down in a four-point colouration scale (see section 3.2.3.), is averaged per male for all male tests or all courtship tests to yield individual scores.

The colouration scores of DH and C males, assessed just after male tests, are not significantly different (Fig. 25: means with standard errors, and Fig. 26: frequency distributions). DL males are, on the average, duller than males in the DH and C line, which, in the course of selection, becomes more pronounced.

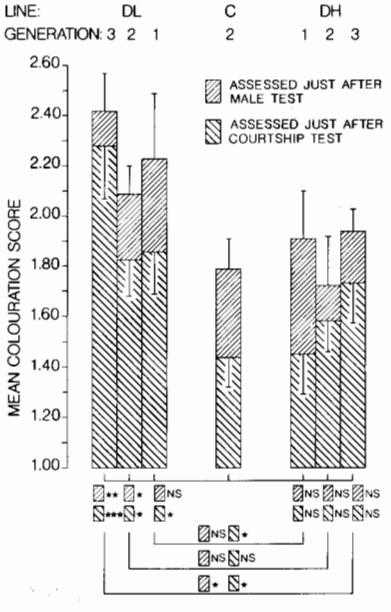

Fig.25. Mean colouration scores, assessed just after the male and courtship tests, for DH, DL and C line males. Lengths of bars represent one standard error of the mean. Distributions of scores tested with Mann-Whitney U test, 1-tailed, H *vs* L (in corresponding generations), H *vs* C-2 and L *vs* C-2: ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

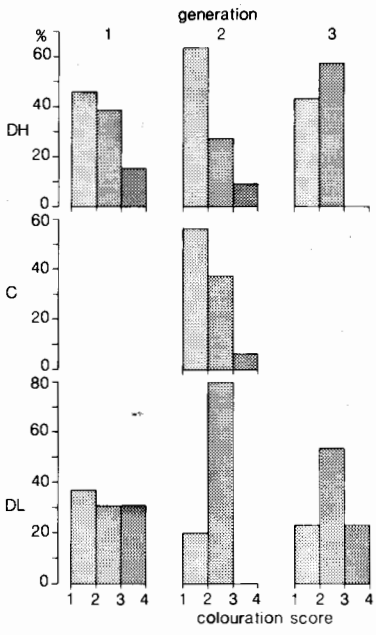

Fig. 26. Frequency distributions of colouration scores, assessed just after the male tests, for DH, DL and C line males.

The intensification of colouration upon exposure to an opponent is more extreme if the opponent is a ripe female (courtship test) than if it is a rival (male test), so the colouration scores assessed just after courtship tests are lower than those assessed after male tests (Figs 25 and 27). Males of the DH line are, on the average, again as bright as C line males, while males of the DL line, on the average, are again duller.

From colouration scores I conclude that selection for a decreased dominance ability is accompanied by a loss of brightness of colouration. This is not a trivial correlation. Because colouration is an important determinant of dominance (BAKKER & SEVENSTER, 1983), selection for decreased dominance ability has obviously acted in favour of phenotypes with a duller colouration. So the decreased dominance ability can be traced to a duller colouration of the males. Selection for high dominance ability has failed probably because with the applied selection procedure colouration of the males can hardly be intensified.

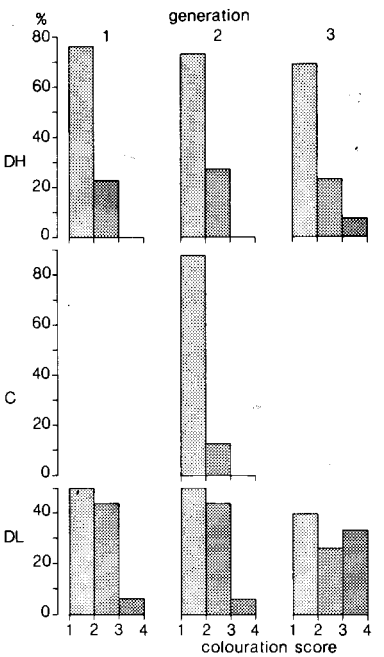

Fig. 27. Frequency distributions of colouration scores, assessed just after the courtship tets, for DH, DL and C line males.

3.6. Other studies involving selection for aggressiveness.

There are but three species that have been used so far in studying aggressiveness by means of selection experiments: the house mouse *Mus musculus*, the domestic chicken *Gallus gallus* and the paradise fish *Macropodus opercularis*. To these the present study adds the three-spined stickleback *Gasterosteus aculeatus* L. In Table 31 the various selection studies on aggressiveness are summarized (see also LAGERSPETZ & LAGERSPETZ, 1974, and MICHARD & CARLIER, 1985, for selection studies in house mice).

One has to be cautious when comparing the results obtained in prior investigations. As can be seen in Table 31, the criterion of selection is quite different, ranging from territorial aggressiveness to aggressiveness in dominance fights and dominance ability. Apart from these different criteria of selection, there exists a huge variation in the behaviour items used as the criterion of selection. Moreover, whereas in most cases selection has been applied to males, in some studies females or both sexes have

TABLE 31. Selection studies on aggressiveness

Organism	Criterion of selection[a]	Number of generations selected (lines[b])	Results: Significant difference between lines in generation:	Results: Selection limit in generation:	Results: Asymmetry of response	Results: Realized heritability	Reference
House mouse (C57B1/10)	♂♂: latency to fighting on neutral area	7 (H, L)	—	0	H = L	—	GINSBURG, 1967
House mouse (C57B1/10)	♂♂: success in fighting on neutral area	7 (H, L)	—	0	H = L	—	GINSBURG, 1967
House mouse (Swiss albino)	♂♂: intensity of fighting on neutral area, assessed with a complex rating scale that included flight	19 (H, L)	2 (H *vs* L)	7 (H + L)	H > L	H + L: 0.36[c]	LAGERSPETZ, 1964; LAGERSPETZ & LAGERSPETZ, 1971
House mouse (wild)	♀♀: aggressiveness in home cage, assessed with a complex rating scale	11 (H, L, C; in dupl.)	2 (H2 *vs* L2) 3 (H1 *vs* L1)	H1: 4[e] H2: 2[e] L1: - L2: -	H < L[d]	H1: 0.12 H2: 0.14 L1: 0.34 L2: 0.46	EBERT & HYDE, 1976; HYDE & SAWYER, 1980
House mouse (wild)	♂♂: attack latency in the border area of the territory	11 (H, L, C)	4 (H *vs* C)	H: 11 L: 0[f]	H > L	H: 0.30 L: -	VAN OORTMERSSEN & BAKKER, 1981
House mouse (ICR albino)	♂♂: frequency and latency of attacks on neutral area combined with reactivity to cutaneous stimulation	4 (H, L, C)	1 (H *vs* L)	H: 0 L: 1	H < L	-[g]	CAIRNS *et al.*, 1983
House mouse (ICR albino)	♂♂: frequency and latency of attacks on neutral area	5 (H, L, C)	3 (H *vs* L)	H: 0 L: 4	H < L	-[g]	CAIRNS *et al.*, 1983
Chicken (White Leghorn)	♂♂ + ♀♀: number of initial paired encounters won on neutral area and ranks in peck-order as supporting evidence	4 (H, L)	2 (H *vs* L)	—	H = L	H + L: 0.18-0.22[h]	GUHL *et al.*, 1960
Chicken (White Leghorn)	♂♂: number of initial pair contests won on neutral area	5 (H, L)	5 (H *vs* L)	—	H = L	H + L: 0.16	CRAIG *et al.*, 1965
Chicken (Rhode Island Red)	♂♂: same as above	5 (H, L)	5 (H *vs* L)	—	H = L	H + L: 0.28	CRAIG *et al.*, 1965
Paradise fish	♂♂ + ♀♀[i]: number of initial paired encounters won on neutral area	5 (H, L, C)	2 (H *vs* L)	H: 0 L: -	H < L	-[j]	FRANCIS, 1984

[a] ♀♀ (or ♂♂) selected according to the results of their brothers (sisters), unless indicated. [b] H = high line, L = low line, C = control line. [c] realized h^2 estimated by MCCLEARN & DEFRIES (1973). [d] doubtful asymmetry, since duplicate C lines differ considerably. H < L with respect to C-0. [e] doubtful limits, since dulicate C lines differ considerably. [f] 4 trials to select for long attack latencies failed because the lines died out after 1 or 2 generations, but a recent attempt appears successful. [g] no h^2-estimates were made. Intraclass correlations for attack frequency: 0.46 and for attack latency: 0.50 in first series (means of generations 1, 2, 3 and 4) and 0.32 and 0.52, respectively, in second series (means of generations 1, 3 and 4). [h] h^2 of % of flock dominated = 0.18, and h^2 of % initial-pair encounters won = 0.22. [i] females were not selected in generations 2 and 4. [j] estimates given for the realized heritabilities are incorrect and rather represent estimates for the responses to selection.

been subjected to selection. A last point of difference is whether the base population is comprised of inbred or wild-caught individuals.

The second caution when comparing different selection studies arises from the nature of the heritability concept. The genetic architecture of a trait is shaped under influence of natural selection (see introduction, section 3.1.) and consequently the history of the base population on which artificial selection will be exerted determines in part the proportion of additive genetic variance and thus the value of h^2. Second, the h^2-value also depends on the conditions under which the selection experiment is carried out and on the extent to which these conditions are standardized. The share of the environmental variance in the total phenotypic variance of the trait selected for and consequently the h^2-value is thereby infuenced. Once can therefore not refer to "*the* heritability of a trait". Heritability is rather a property of the trait in a certain population under a given set of conditions.

If we review Table 31 with these restrictions in mind and compare the h^2-values with those assessed in the present study (see sections 3.3.2., 3.4.2. and 3.5.2.), the rather large h^2-values in all selection studies on aggressiveness become apparent; they indicate a relatively large contribution of additive genetic variance under the environmental conditions used in the various studies. Unfortunately, from the h^2-values alone, assertions about the genetic architecture remain highly speculative since the magnitude of the dominance variance is unknown. In the case of the three-spined stickleback the magnitudes of the two main components of the genetic variance can be estimated, since also full sib correlations for the different aggression scores in the base population are known (see section 2.6.2.). This subject will be discussed in section 4.4.

Another striking point is the degree and direction of asymmetry of the response to two-way selection for aggressiveness. Whereas selection in the three-spined stickleback shows less response in upward direction (see sections 3.3.1., 3.4.1.1. and 3.5.1.), like in the paradise fish (FRANCIS, 1984), that of the house mouse shows the reverse in at least some studies (LAGERSPETZ, 1964; LAGERSPETZ & LAGERSPETZ, 1971; VAN OORTMERSSEN & BAKKER, 1981) and in the chicken there is no question of asymmetry at all (GUHL *et al.*, 1960; CRAIG *et al.*, 1965). If these asymmetrical responses are to be interpreted in terms of different proportions of additive genetic variance in opposite directions, an interpretation that can be partly justified for the stickleback (see section 4.4.), then in the case of the three-spined stickleback (at least in the base population of the present study) natural selection should have favoured enhanced ag-

gressiveness in certain situations (viz. territorial aggressiveness and dominance ability in males). In some house mouse populations, however, selection seems to have acted in favour of a diminished aggressiveness in certain situations, possibly a consequence of the deme-structure of house mouse populations. The obvious symmetry in response to bidirectional selection for dominance ability in chickens suggests intermediate gene frequencies of the genes involved in this phenotype.

A final topic of this section concerns the increase in h^2-values from the selection studies for aggressiveness in chickens to those in house mice, and from the latter to those in sticklebacks. This is very likely a reflection of the degree to which environmental conditions can be standardized in the different species. Chickens were reared socially up to 4 or 5 months of age, in house mice social isolation (after weaning) was applied in some studies, and in three-spined sticklebacks juveniles were isolated very early in their development. Social isolation is an important means of standardizing environmental variables. The differences in h^2-values between the species are therefore likely to reflect for the most part differences in the relative amount of environmental variance.

3.7. Summary.

With the fish of the base population which were tested for their aggressiveness in different situations, six artificial selection lines were founded, with aggressiveness serving in each of these situations as a criterion of bidirectional selection. An unselected control line was maintained. The lines were selected according to:

a. High or low juvenile aggression score in both juvenile males and females: juvenile aggression lines.
b. High or low territorial aggression score in males and high or low female aggression score in females: territorial aggression lines.
c. High or low dominance ability in males: dominance lines.

a. *Juvenile aggression lines.*

Selection for juvenile aggressiveness is successful in downward direction for both sexes. The response to selection in upward direction is doubtful. This partly can be ascribed to limitations of breeding, because of extreme aggressiveness of females and because of reduced viability of fertilized eggs. Selection for juvenile aggressiveness is accompanied by a change in developmental rate; juvenile aggression starts on the average at an earlier age in young of the high line than in those of the low line. Furthermore, age at sexual maturity is positively correlated with juvenile aggressiveness across the generation means. A most intriguing matter is the course of juvenile aggressiveness as a function of age; juvenile aggressiveness rises up to an age of 10-11 weeks (after fertilization) and then declines. The decline cannot be ascribed to habituation to the test situation.

b. *Territorial aggression lines.*

The response to bidirectional selection for territorial aggressiveness in reproductive males is highly asymmetrical; the high line shows no response at all, while selection in

downward direction is highly successful. The absence of response in upward direction cannot be attributed to specific causes. In reproductive females, on the contrary, aggressiveness is as easily enhanced as it is lowered. In both sexes aggressiveness is positively correlated with responsiveness to the test stimulus across the generation means. Males of the low line prefer to build their nest closer to the front pane than males of the high line. The incidence of ripeness during the female tests tends to be higher in the high line than in the low line, especially when the females grow older. Non-ripe females of the low line respond on the average less to the female test than ripe ones do.

c. *Dominance lines.*

Inter-line dominance tests show that the difference in mean dominance ability between the high and low dominance line increases with the progress of selection. Inter-line dominance tests with control line males reveal that this difference can very likely be ascribed to a gradually decreasing mean dominance ability of the low line. The lack of response in the high line is accompanied by a severe reduction in viability of fertilized eggs. Further, the asymmetry of response is reflected in the decreasing brightness of colouration of males of the low line, while in high line males the reverse change is not observed. This is additional evidence for the importance of the brightness of colouration as a determinant of dominance in three-spined stickleback males. Size of the males is not correlated with the rank of a male in the linear order of dominance. Dominance in the stickleback is compared to nip-dominance hierarchies in other fish species.

The present study has clearly shown that, under the applied experimental conditions, the variation of juvenile aggressiveness, territorial aggressiveness, female aggressiveness and dominance ability can to a considerable extent be ascribed to additive genetic variation. The combined two-way responses yield h^2-estimates of at least 0.30 for aggressiveness in the different situations. The realized heritabilities in the single selection lines range from 0 (no response) to at least 0.60. The selection results are discussed in comparison with other selection studies on aggressiveness.

4. Correlated responses

4.1. Introduction.

In the previous chapter the results of selection experiments were discussed. Selection was exerted in each case on one character, the characters being aggressiveness in a number of different situations. These different criteria of selection are correlated with each other to various degrees (see section 2.5.2.). 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. Specifically, the genetic correlation is the correlation between the additive genetic values for the two characters on the same individuals in a population and the environmental correlation is the correlation of environmental deviations together with non-additive genetic deviations (see *e.g.* FALCONER, 1981). In this chapter the genetic correlation is dealt with.

The genetic cause of correlation is chiefly pleiotropy, which results in a permanent correlation between characters. Transient correlation may be due to temporary associations between genes at different loci (linkage

disequilibrium) as a consequence of recent admixture of populations. The degree of correlation arising from pleiotropy expresses the extent to which the variation of two characters is influenced by common (pleiotropic) genes. Dealing with quantitative characters, the correlation resulting from pleiotropy is the overall effect of all the segregating genes that affect both characters. Some genes may increase both characters and tend to cause a positive correlation, while others may increase one character and decrease the other and so tend to cause a negative correlation. The overall effect may be an absence of correlation, which does not necessarily indicate an absence of pleiotropy.

In selection studies the degree of genetic correlation may be deduced from the changes in characters not directly selected for (correlated responses), as compared with the response in the criterion selected for. In this chapter a number of correlated responses are reviewed, with the emphasis on aggressive behaviour. Two central questions are considered: Are there common genetic influences on the variation of aggressiveness in the different test situations? and: Are the different elements of aggressive behaviour in a particular situation all changing in the same direction when selection is exerted on one of them? Changes of non-aggressive behaviour resulting from selection for aggressiveness will be treated in a subsequent paper. A final topic of this chapter is the hormonal influences on aggressiveness. This is discussed in the light of data on kidney size and size of the testes of reproductive males of the different genetic lines from the present study. The appendix, which summarizes the major responses and correlated responses in generation 3, may be used to facilitate the reading of this chapter.

4.2. Material and methods.

4.2.1. *Correlated responses of dominance ability.*

To assess the correlated response of dominance ability in males of the juvenile and territorial aggression lines, a number of randomly chosen combinations of third generation males of these lines and of the dominance lines were tested for dominance after the completion of the usual testing-scheme. The deviation from random (50% gain or loss) of the dominance test outcomes for each line indicates their difference in mean dominance ability with that of the DH or DL line.

4.2.2. *Aggression in groups of juveniles.*

In addition to the individually isolated fish, groups of 15 full sibs each were made 3 weeks after fertilization. Of the juvenile aggression lines in generation 3 groups of 5 full sibs each were also separated. All groups were kept in tank compartments of $34.5 \times 40 \times 50$ cm, in which the back wall and one side wall was planted with a row of longleafed plants. Each tank was illumintated by a 100 Watt bulb about 20 cm above the water surface. The light regime, temperature and diet were the same as for their individually isolated full sibs (see section 2.1.1.).

From day 35 after fertilization the aggression in groups of juveniles of C-2, JH-3 and JL-3 was weekly scored as the total number of bites and bumps in the group during a 5 min period. This measure of aggression I call the flashnumber of a group, since bites and bumps in a group of juveniles often are of very short duration and in fact are recognized by the "flashing" movements that accompany such behaviour. This means that attacks which nearly end in a bite or bump are also included in the flash-number.

In addition to flash-number, the dispersion of the young in the group during the same period was recorded on a four-point dispersion scale:

1. All fish are aggregated in a school.
2. Part of the fish are aggregated in a school, others are moving at smaller and greater distance from the school.
3. The fish are scattered all over the tank.
4. Some or most fish are defending a territory, scattered throughout the tank.

At the end of the 5 min observation period the reaction of the group to a frightening stimulus was assessed. The frightening stimulus consisted of the sudden appearance of the observer's head, whose nose was pressed against the glass wall of the tank. The reaction of the group to such a frightening stimulus can be placed on a three-point fright scale:

1. The fish hardly react to the frightening stimulus.
2. The fishes' movements freeze.
3. The fish dart between the plants, stay there motionless and sometimes dash about the tank for a few seconds.

The scores obtained on the dispersion scale and the fright scale during the juvenile phase (6th up to and including the 16th week after fertilization, that is before one of the group-members attained sexual maturity) are averaged per group to yield dispersion scores and fright scores, thus facilitating comparisons between groups.

4.2.3. *Kidney size and testis size.*

In generation 4 the kidney size and testis size of reproductive males of the selection lines and of the C line was measured 7-8 months after fertilization. The males were decapitated, their body cavity opened and their kidneys and testes excised. Kidneys and testes were measured under a binocular with the aid of an ocular micrometer.

The paired kidneys of the stickleback lie under the vertebral column. In reproductive males the kidneys have been enlarged to pale pink, hypertrophied organs, that produce mucus for nestbuilding. In the analysis of kidney size only data of reproductive males with hypertrophied kidneys were used. A few with non-hypertrophied kidneys were disregarded. Since the mean kidney size of isolated and non-isolated C-4 males did not differ significantly, the data of males with a different previous social history were combined. In C-4 there is a significant, positive correlation between body length (standard length in cm) and kidney size ($1 \times w$ in mm^2, *i.e.* the approximate area of the largest longitudinal section of the fused kidneys; see Fig. 28) (regression line: $\hat{y} = 16.34x - 42.98$, $r = 0.65$; t test, 2-tailed, df 37, $p < 0.001$). To correct this, both the length and the width of the kidneys are divided by the male's body length. The thus obtained kidney size index is independent of the male's body length ($\hat{y} = -0.06x + 1.78$, $r = -0.09$; $p > 0.20$).

The paired testes of the male lie in a ventro-lateral position in the body cavity and are pigmented black in a greater or less degree. Because of the more cylindrical form of the testis, size measurements were made in three dimensions (Fig. 28). The height and width were measured at the midpoint, since the dimensions of the testis increase in the anterior direction. The proportions of both testes of an individual do not deviate much from each other and were therefore averaged. The testes of isolated C-4 males tended to be larger than those of their non-isolated brothers: isolated ♂♂ ($n = 21$) mean $1 \times w \times h = 8.10$ mm^3 and non-isolated ♂♂ ($n = 18$) mean = 6.37 mm^3. Although this difference is not significant (Mann-Whitney U test, 2-tailed, $p > 0.20$), values corrected for differences in the

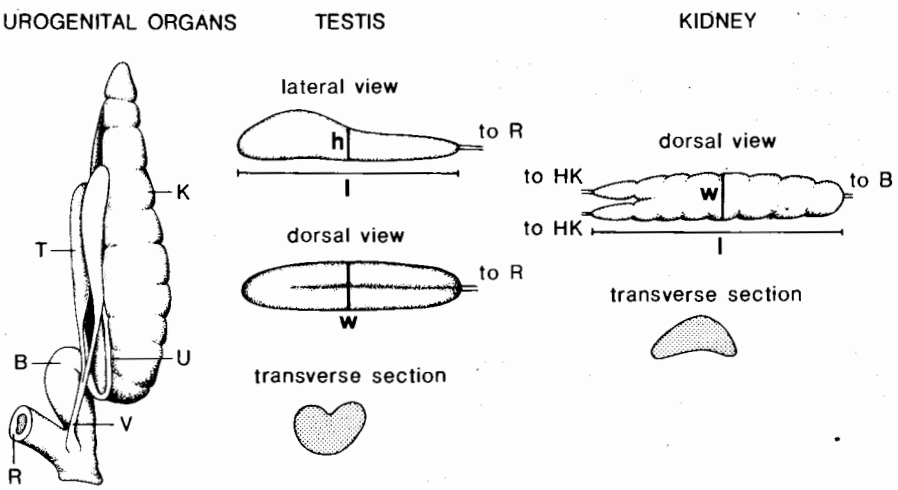

Fig. 28. Urogenital organs of the reproductive male stickleback, and size measures for the testis and kidneys (l = length, h = height, w = width). B urinary bladder, HK head kidney, K kidney, R rectum, T testis, U ureter, V vas deferens. Left figure after IKEDA, 1933.

male's body length reveal a significant difference between isolated and non-isolated males (Mann-Whitney U test, 2-tailed, $p<0.05$). Contrary to kidney size, where such a discrepancy between isolated and non-isolated males was not present, the analysis of testis size is treated separately for isolated and non-isolated males. Testis size is, like kidney size, positively correlated with body length (isolated C-4 ♂♂: $\hat{y} = 7.25x\text{-}24.75$, $r = 0.62$; t-test, 2-tailed, $p<0.01$, and non-isolated C-4 ♂♂: $\hat{y} = 9.18x\text{-}36.27$, $r = 0.84$, $p<0.001$). To correct this, the approximate mean volume of both testes ($l \times w \times h$ in $mm^3 \times 100$) is divided by the length of the male cubed (in cm^3). In isolated C-4 males the thus calculated testis size index is independent of the male's body length ($\hat{y} = 2.00x\text{-}1.08$, $r = 0.20$; $p>0.20$), but in non-isolated C-4 males a substantial correlation remains even after this correction ($\hat{y} = 4.39x\text{-}14.37$, $r = 0.65$; $p<0.01$). The reason for this correlation is unclear. Possibly the testes' weight, relative to the male's body weight, would have been a better testis size index.

4.2.4. *Methods of quantitative genetics.*

Genetic correlations can be estimated in three ways. The first one is based on the resemblance of related individuals and is thus analogous to the estimation of heritability (see section 2.1.4. for h^2-estimates from the resemblance of full sibs) (*e.g.* FALCONER, 1981). If a set of data permits h^2 estimation, genetic correlations may be estimated from the same dataset when two or more characters are measured on each individual. Instead of computing the components of variance of one character from an analysis of variance, the components of covariance of the two characters are computed from an analysis of covariance. The genetic correlation can also be estimated from the responses to selection in a manner analogous to the estimation of the realized heritability (see section 3.2.4.). If selection is exerted on one character and the correlated response of the other character is measured, then the heritabilities of both characters must be known in order to compute

the genetic correlation. The genetic correlation can also be computed without knowledge of the heritabilities of the characters. In that case a double selection experiment has to be carried out, so that both the direct and correlated responses of each character can be measured. A joint estimate of the genetic correlation can then be obtained from the following equation:

$$r_A^2 = \frac{CR_X}{R_X} \frac{CR_Y}{R_Y}$$ (FALCONER, 1981)

where r_A symbolizes the genetic correlation between X and Y, R the response and CR the correlated response with subscripts X or Y according to the character referred to. This last method is used in the present paper.

Estimates of genetic correlations are, however, often subject to large sampling errors. Add to this the limited number of selected generations in the present study and it is evident that the data from the present selection experiments can only yield rough estimates of genetic correlations. In this chapter a qualitative approach is therefore emphasized, although some attention will be given to the estimation of genetic correlations.

4.3. Correlated responses of the different criteria of selection.

Fish of a particular selection line have not only been scored for aggressiveness in the particular situation in which they were selected, but also for aggressiveness in other situations (criteria of selection in other selection lines). Therefore within one selection line the response (R) to selection for aggressiveness in one situation can be compared to the correlated responses (CR) of the other criteria of selection. In this section the different criteria of selection are compared pairwise in the two sets (H and L) of the selection lines concerned. The extent to which selection for aggressiveness in one situation affects aggressiveness in another situation, and *vice versa*, is a measure for the genetic correlation of aggressiveness in both situations.

First, the aggression of females during the two aggression tests (juvenile and female test) is compared in section 4.3.1. in order to deduce the genetic correlation of juvenile and female aggression scores. In section 4.3.2. the same comparison is made for males to estimate the genetic correlation of their juvenile and territorial aggression scores. Section 4.3.3. is devoted to dominance ability. The correlated responses of aggression during the aggression tests in both sexes of the dominance lines and the dominance ability of males of the juvenile and territorial aggression lines is analysed. Finally, in section 4.3.4. some genetic correlations are estimated quantitatively.

4.3.1. *Juvenile and female tests on females of the territorial and juvenile aggression lines, respectively.*

When the mean juvenile aggression scores of juvenile females are compared with their mean female aggression lines, then the similar courses of the responses and correlated responses with proceeding selection are striking (Fig. 29; cf. left and right top graphs and cf. left and right bottom graphs). This means that many genes (if not all) effecting variation of aggressiveness in the two 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 the variation of aggressiveness in female does not change with sexual maturity.

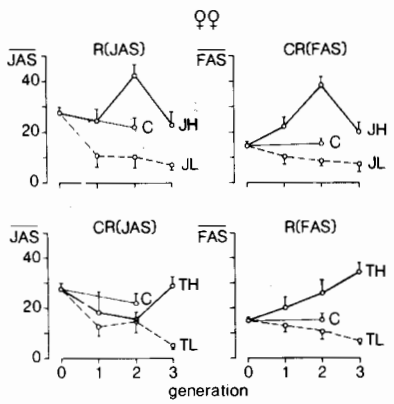

Fig. 29. Responses (R) and correlated responses (CR) of both juvenile (JAS) and female aggression score (FAS) of females. Top graphs: juvenile aggression lines, bottom graphs: territorial aggression lines. Lengths of bars represent one standard error of the mean.

The correlated responses of FAS in the juvenile aggression lines (Fig. 29 right top graph) are more striking than those of JAS in the territorial aggression lines (Fig. 29 left bottom graph), especially in the high line. This is expected, since in the JH and JL line both parents have been selected for JAS. In chapters 2 and 3 sufficient evidence has been accumulated in support of an identical genetic influence on the variation of juvenile aggressiveness in juveniles of both sexes. In the territorial aggression lines, however, males were selected according to their TAS and females selected according to their FAS. If variation of FAS is assumed to be effected by the same genetic factors as variation of JAS, an assump-

tion that seems to be justified by the above results, then the extent to which males and females of the TH and TL line have been selected for common genetic factors depends on the genetic correlation between JAS and TAS in males. Anticipating the results to be discussed in the next section, this genetic correlation in males is far from absolute. So selection in the territorial aggression lines probably has been less intense than in the juvenile aggression lines and as a consequence the correlated responses are weaker.

Another factor that may have influenced the correlated responses of juvenile aggressiveness in females of the territorial aggression lines is the unexpectedly low responsiveness in the juvenile tests (mean responsiveness is about 25%). The low responsiveness causes a reduction of sample size due to non-responders and, considering the course of juvenile aggressiveness with age (see section 3.3.3.), probably leads to an underestimation of the juvenile aggression scores of low responders. The low mean responsiveness could therefore have contributed to the absence of a correlated response of JAS in TH-1 and TH-2 (bottom left graph in Fig. 29; TH-1 and TH-2 *vs* C-2, Mann-Whitney U test, 2-tailed, $p > 0.10$).

The responsiveness in the juvenile tests and the mean JAS increases in generation 3 (mean responsiveness in TH-3: 36.3% *vs* 17.5% and 22.2% in TH-1 and TH-2 juvenile females respectively). The increase of mean JAS is not yet significant compared to C-2 (Mann-Whitney U test, 1-tailed, $p > 0.05$), but the difference in mean JAS between TH-3 and TL-3 females is highly significant (Mann-Whitney U test, 1-tailed, $p < 0.001$), and thus in agreement with the response to selection for FAS. For a more definite quantitative judgement of the genetic correlation more selected generations are certainly needed. In Fig. 30 the correlated responses of FAS and JAS in females of the juvenile and territorial aggression lines respectively are again presented as frequency distributions of aggression scores.

The low responsiveness in juvenile tests of TH and TL females was contrary to expectation, since in these lines (and in the juvenile aggression lines as well) some selection pressure must have been exerted against poor responders, as non-responders were excluded as parents and, with comparable aggression scores, females that responded well were given priority over those that responded poorly. Furthermore, responsiveness in the female test is positively correlated with that in the juvenile test (base population: $r = 0.55$; t test, 2-tailed, df 47, $p < 0.001$). On the other hand, the selection pressure against poor responsiveness was probably greater in the juvenile aggression lines than in the territorial aggression lines, since in general juvenile females responded less well in the juvenile test than adult females did in the female test. This does, however, not explain the low responsiveness in the juvenile tests of TH and TL females. Some factors that could have con-

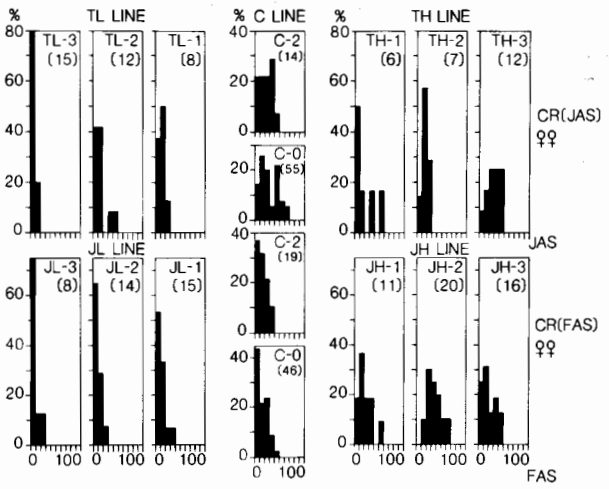

Fig. 30. Frequency distributions of juvenile (JAS) and female aggression scores (FAS) of females from the territorial aggression lines and the juvenile aggression lines, respectively. Numbers of individuals tested are shown in parentheses. CR = correlated response.

tributed to this low responsiveness can be suggested, such as the lack of a correlation between responsiveness and aggressiveness in juveniles (base population: $r = 0.13$; t test, 2-tailed, df 53, $p > 0.20$), or a negative contribution to the responsiveness on the father's side (see next section).

In section 3.4.1.2. it was shown that in the territorial aggression lines females of the low line are less often in a ripe condition than those of the high line, although this difference was not significant during the first month of sexual maturity. In the juvenile aggression lines the difference in reproductive capacity between high and low line females is more pronounced: JL females are significantly less often ripe than JH females (first four, valid or invalid, female tests, JH ripe:non-ripe = 80:112 and JL 41:123; χ^2 test, 1-tailed, $0.0005 < p < 0.005$) (Table 32). Selection for juvenile aggressiveness is clearly accompanied by a change in the incidence of ripeness at the adult stage. Differences in

TABLE 32. Incidence of ripe females during the first four female tests, separated according to valid and invalid tests, in the JH and JL line

Generation	Incidence of ripe ♀♀ in first 4 female tests							
	JH				JL			
	Valid		Invalid		Valid		Invalid	
	n	%	n	%	n	%	n	%
1	9	32.1	9	36.0	10	28.6	13	38.2
2	40	56.3	3	27.3	8	24.2	2	6.5
3	12	28.6	7	46.7	4	22.2	4	30.8
Total	61	43.3	19	37.3	22	25.6	19	24.4

food supply and size differences between JH and JL females can be ruled out as causal factors (see WOOTTON, 1973): standard lengths, assessed 8-9 months after fertilization, of JH-3 5.3 ± 0.4 (n = 16) and of JL-3 5.5 ± 0.3 (n = 10); Mann-Whitney U test, 2-tailed, $p > 0.10$. The fact that the accompanying change in the incidence of ripeness is less pronounced in TH and TL line females can probably be ascribed to the lower intensity of selection in those lines compared to the juvenile aggression lines, as outlined earlier in this section.

4.3.2. *Juvenile and male tests on males of the territorial and juvenile aggression lines, respectively.*

In males, the genetic correlation between the aggression scores obtained in the two aggression tests, viz. the juvenile and male test, is much smaller than in the females (Fig. 31: mean scores and Fig. 32: frequency distributions of aggression scores). The only feature which might point to some genetic correlation between juvenile and territorial aggression scores is the systematically lower means of the correlated aggression scores in both low lines compared to the high lines. However, the differences between the high and low lines nowhere reach significance.

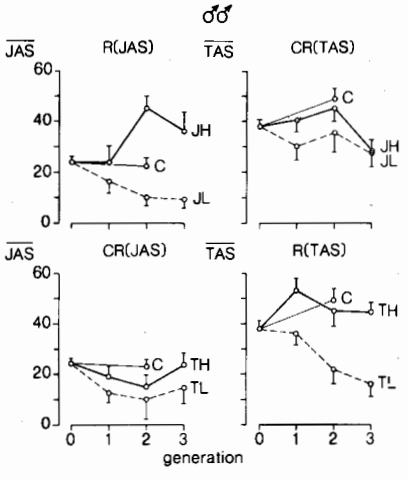

Fig. 31. Responses (R) and correlated responses (CR) of both juvenile (JAS) and territorial aggression score (TAS) of males. Top graphs: juvenile aggression lines, bottom graphs: territorial aggression lines. Lengths of bars represent one standard error of the mean.

It is not surprising to find that the mean level of territorial aggression in JH males does not exceed the control level (Fig. 31 right top graph). This is also true in the TH lines, where a selection pressure in the

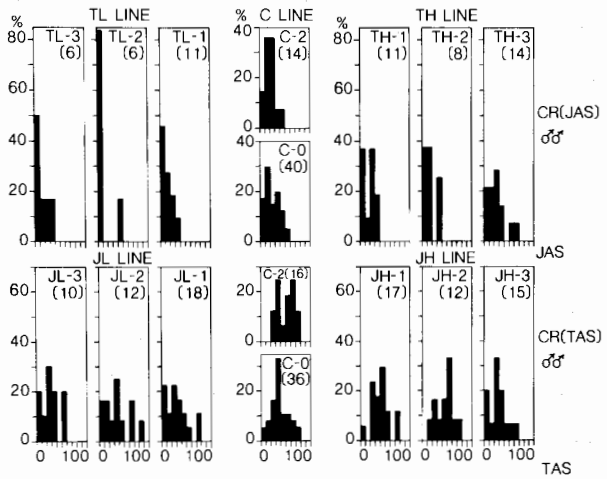

Fig. 32. Frequency distributions of juvenile (JAS) and territorial aggression scores (TAS) of males from the territorial aggression lines and the juvenile aggression lines, respectively. Numbers of individuals tested are shown in parentheses. CR = correlated response.

enhanced direction was exerted (Fig. 31 right bottom graph). Increasing the TAS is apparently restricted (for possible causes see section 4.4.). The decline of the mean TAS in JH-3, which is significant compared to C-2 (Mann-Whitney U test, 2-tailed, $p<0.02$), is more difficult to explain. It is true that the mean JAS also shows a drop in JH-3, but not that extreme. This decrease of mean TAS may be partly explained by another nest-site choice of JH-3 males (see below). The level of territorial aggression in the JL line is decreased (mean TAS of JL-1, JL-2 and JL-3 *vs* C-2: Mann-Whitney U test, 1-tailed, $p<0.01$, $P<0.05$ and $p<0.01$ respectively). This might point to a certain degree of genetic correlation between JAS and TAS in JL males. It is, however, likely that differences in nest-site choice between the lines cause an overestimation of this genetic correlation (see below).

Because of the experimental set up in the present study, the location of the nest is important to judge the differences in territorial aggressiveness. Males of the JL line show a distinct preference to build their first nest away from the front pane; JL-(1+2+3) front:back = 12:24, χ^2-test, 2-tailed, $0.02<p<0.05$ (Fig. 33). In this respect JL males do not resemble TL males, which on the contrary preferred to nest in the front-half of their tanks (see section 3.4.1.1.). Males of the first two generations of the JH line show no preference as to their nest-site choice; JH-(1+2) front:back = 13:13. The difference in nest-site choice between JH and JL line males may have been contributed to the lower mean TAS in JL-1 and JL-2, and thus to the genetic correlation between JAS and TAS in JL males. JH-3 males preferred, however, to build their nest in the planted areas in

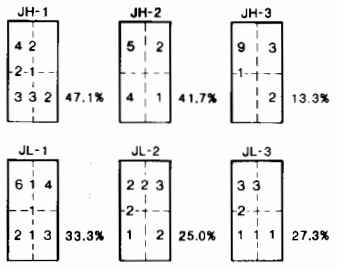

Fig. 33. Locations of first nests of JH and JL line males, and % of first nests in the front half of tanks (excluding border area between front and back half).

the back-half (front:back = 2:12, binomial test, 2-tailed, $p = 0.012$), and so the opponent was offered at greater average distance from their nest than in the two preceding generations. The low mean TAS of JH-3 males may therefore be at least partly attributed to this different nest-site choice.

In TH males the correlated character of JAS shows, like in TH females (see previous section), a decline, though insignificant, in the first two selected generations, although these males have been indirectly selected for an increased juvenile aggressiveness (at least through selection for high female aggression scores of their mothers). Like in TH females (see section 4.3.1.) this initial decrease probably results from the low responsiveness of TH males in the juvenile tests (27.7% and 22.0% in TH-1 and TH-2 respectively).

Since responsiveness in the male test is uncorrelated with responsiveness in the juvenile test, TH males were not selected for high responsiveness in the juvenile test; in the base population nearly all males always reacted in the male test, while responsiveness in the juvenile test was quite variable (see Fig. 4). Furthermore, like in juvenile females (see section 4.3.1.), responsiveness and aggressiveness in the juvenile phase are uncorrelated (base population: $r = 0.30$; t test, 2-tailed, df 35, $0.05 < p < 0.10$). Why TH-1 and TH-2 juvenile males show a lower responsiveness than C-2 juvenile males (45.7%) remains, however, obscure.

TH-3 males responded, however, much better in the juvenile test (mean responsiveness 59.4%), probably because of the gradually increasing responsiveness in the female test by their mothers. The correlated response of JAS in TL males suggests a certain degree of genetic correlation between JAS and TAS, like in the JL line. Due to low numbers (for reasons see section 3.4.2.), only the mean JAS of TL-2 males deviates significantly from the C-2 level (Mann-Whitney U test, 2-tailed, $p < 0.05$). Certainly more selected generations are needed to confirm the trend.

4.3.3. *Aggression tests in the dominance lines and correlated responses of dominance in the other selection lines.*

In the dominance lines females were not selected directly and in males the response to selection for dominance ability was measured as the interline difference in mean dominance ability. The analysis of the genetic correlation between dominance ability and aggressiveness during the other behavioural tests will therefore be somewhat different from the pairwise comparisons in the previous two sections. The correlated responses of aggression in the aggression tests on both sexes of the two dominance lines and the correlated responses of dominance ability in males belonging to the juvenile and territorial aggression lines will be discussed in succession.

4.3.3.1. Aggression in males of the dominance lines.

The correlated responses of JAS and TAS in DH and DL males are shown in Fig. 34A (mean scores and standard errors) and in Fig. 35 (frequency distributions of scores). Selection for dominance ability is hardly accompanied by changes in juvenile aggressiveness and territorial aggressiveness. When the DH line is compared to the C-2 level no significant changes are found (Mann-Whitney U test, 2-tailed, $p > 0.10$). In the DL line there is a slight tendency towards a decreased mean JAS and a decreased mean TAS. The decrease, however, in no case reaches the level of significance compared to C-2 or DH males (Mann-Whitney U

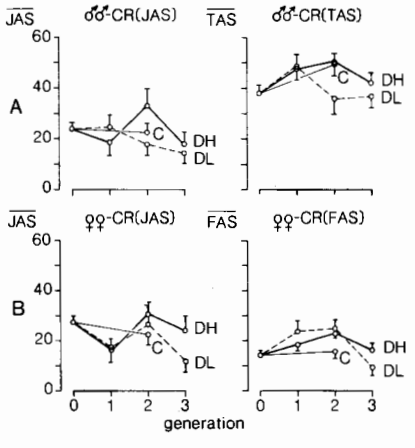

Fig. 34. A: correlated responses (CR) of juvenile (JAS) and territorial aggression score (TAS) of DH and DL males and, B: CR of JAS and female aggression score (FAS) of DH and DL females. Lengths of bars represent one standard error of the mean.

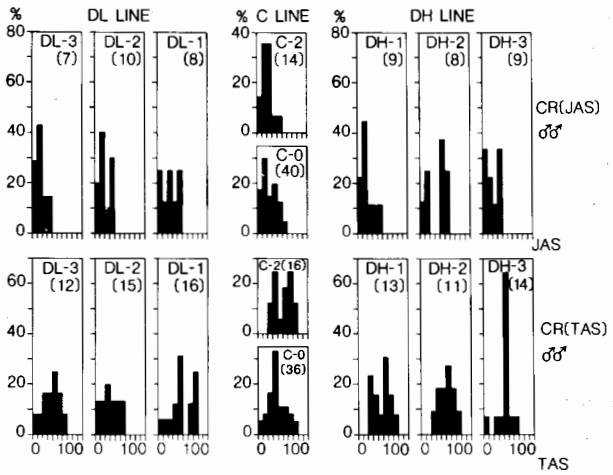

Fig. 35. Frequency distributions of juvenile (JAS) and territorial aggression scores (TAS) of males from the dominance lines. Numbers of individuals tested are shown in parentheses. CR = correlated response.

test, 2-tailed, $p > 0.10$). I therefore conclude that the genetic correlation between dominance ability and juvenile aggressiveness and between dominance ability and territorial aggressiveness is very weak, if present at all. FRANCIS (1984) arrived at the same conclusion for dominance and aggressiveness in *Macropodus opercularis*.

It cannot be entirely excluded that a weak genetic correlation exists between dominance and TAS, since there is a slight difference in nest-site choice between DH and DL males which might have reduced the difference in mean TAS between the dominance lines. As compared to low line males, DH males tend to build their first nest away from the front pane; DL-(1+2+3) front:non-front = 26:19 *vs* DH-(1+2+3) 14:25, χ^2 test, 2-tailed, $0.05 < p < 0.10$ (Fig. 36). This leads to a significant difference in the positions of the nests between DH and DL males during the valid male tests (nests during valid male tests, DH-(1+2+3) front:non-front = 53:101 and DL-(1+2+3) 97:82; χ^2 test, 2-tailed, $p < 0.001$).

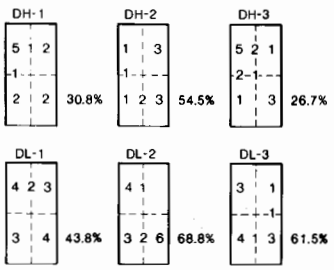

Fig. 36. Locations of the first nests of DH and DL line males, and % of first nests in the front half of tanks (excluding border area between front and back half).

4.3.3.2. Relation between aggression, responsiveness and nest-site choice.

The question arises whether differences in the choice of nest-site reflect differences in territorial aggressiveness. If males of the territorial aggression lines and the dominance lines are considered as to their nest-site choice and their territorial aggressiveness, then it is tempting to assume a relationship between the two variables: territorial aggressiveness is (or tends to be) higher in the TH and DH line compared to the low lines and males of both high lines show the same tendency to nest in the back (see section 3.4.1.1. and 4.3.3.1.). The assumption is, however, contradicted by the reverse relationship in the juvenile aggression lines: here the low line, which tends to have lower territorial aggression scores, shows the preference to nest in the back, at least in the first two generations (see section 4.3.2.).

In searching for factors shared by males of the TL and DL line, which showed a more or less pronounced preference to nest in the front-half (see section 3.4.1.1. and 4.3.3.1.), the low responsiveness in the male tests on these males was striking (Fig. 37). This suggests that differences in nest-site choice may be explained by differences in responsiveness rather than by differences in territorial aggressiveness (cf. JENNI *et al.*, 1969, who have shown that "shyness" infuences nest-site selection).

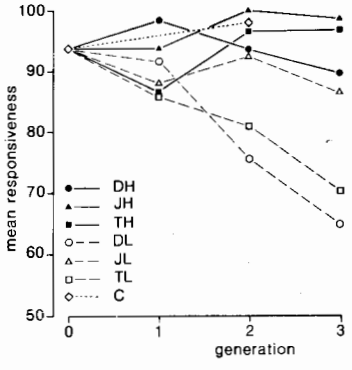

Fig. 37. Mean responsiveness (expressed as mean % valid tests) of the different selection lines in the male tests during the progress of selection.

One of the variables that influences responsiveness in male tests is territorial aggressiveness: as in females (see section 3.4.1.2.), there exists a distinctly positive correlation between responsiveness and TAS across the generation-means of the two territorial aggression lines (Fig. 38:

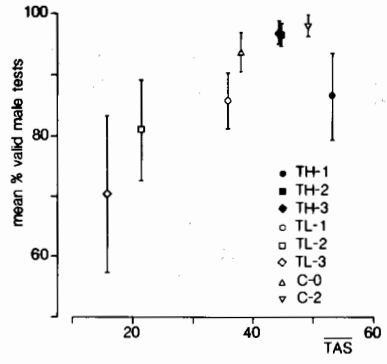

Fig. 38. The correlations of the mean responsiveness during male tests (expressed as mean % valid tests) with the mean territorial aggression score ($\overline{TAS}$) in the TH, TL and C line. Lengths of bars represent two standard errors of the mean.

$\hat{y} = 0.60x + 66.15$, $r = 0.81$; t test, 2-tailed, $0.01 < p < 0.02$). However, if the three selected generations of all six selection lines are considered, then the correlation across the generation-means is less pronounced ($r = 0.51$; t test, 1-tailed, $0.01 < p < 0.025$). Responsiveness is evidently influenced by more variables than merely territorial aggressiveness, and one is tempted to label these "shyness". However, such a label does not explain things and I therefore prefer to use the more objective term of responsiveness, in which these variables are included. A low responsiveness means a long meet latency, which may result from a number of causes, *e.g.* low territorial aggressiveness and/or high sensitiveness to frightening stimuli. The different causes are difficult to separate and are probably correlated with each other to some degree. They all result, however, in a diminished responsiveness of the male in the male test. The same factors that cause low responsiveness in TL and DL males (although they may be partly different in both lines) may cause them to build their nest closer to the front pane. That is, TL and DL males may be more susceptible to disturbances, of whatever kind, and their nest-building behaviour may therefore be inhibited when observations are being made, so that they start nest-building in the undisturbed hours of artificial light. The preference of undisturbed TL and DL males to nest in the front-half can then be interpreted in terms of a "repelling" influence of the vegetation in the back-half (VAN IERSEL, 1958; JENNI, 1972); such males accept the vegetation as a boundary of their territory. The fact that during the invalid, discarded tests of TL-3 males the nests are located significantly closer to the front pane than during the valid male tests (TL-3 valid tests,

front:non-front = 11:13 and invalid tests 13:2; χ^2 test, 1-tailed, $0.01 < p < 0.025$) seems to sustain this view.

The nest-site choice of males which start and go on with nest-building during the hours of observation is the resultant of several influences, which are inextricable with the data of the present study, such as a repelling influence of the front pane resulting either from disturbances during the hours of observation, or from the presentation of opponents at the front pane, a repelling influence of the vegetation, and an attractive influence of single plants. (VAN IERSEL, 1958; VAN DEN ASSEM, 1967; JENNI *et al.*, 1969; JENNI, 1972).

4.3.3.3. Aggression in females of the dominance lines.

Since selection for dominance in males had hardly any effect on juvenile aggressiveness (see section 4.3.3.1.), this is also expected for the female's aggressiveness during the juvenile and female tests. The more so as females of the dominance lines were not directly selected for dominance because of the absence of a convenient measure for dominance ability in females (see section 2.4.3.). The relevant data are presented in Fig. 34B (mean scores with standard errors) and in Fig. 39 (frequency distributions of scores). Females of the high and low dominance line do not differ with respect to their mean JAS and FAS; all differences between mean scores of DH- and DL females in comparable generations are not signifi-

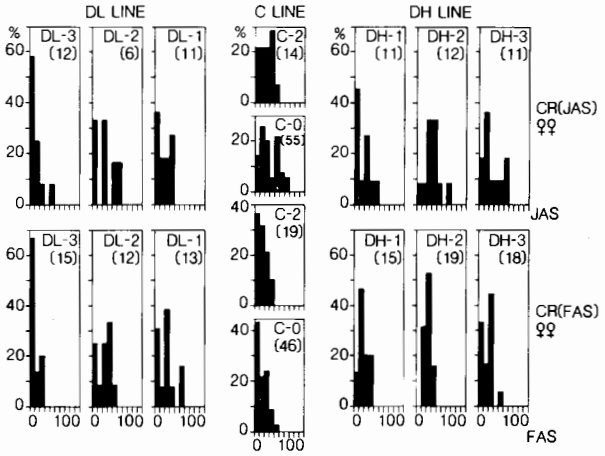

Fig. 39. Frequency distributions of juvenile (JAS) and female aggression scores (FAS) of females from the dominance lines. Numbers of individuals tested are shown in parentheses. CR = correlated response.

cant (Mann-Whitney U-test, 2-tailed, $p > 0.10$). Compared to the C-2 level there are, however, some significant deviations at the 5% level (JAS: DL-3; FAS: DH-2, DL-2, DL-3), which cannot be explained. There are no systematic trends, so they may be considered random fluctuations.

The effect of selection for the males' dominance ability on aggressiveness of females belonging to the dominance lines, as measured with the juvenile and female test, is, strictly speaking, not covered by the term "correlated response", since "correlated characters" are defined as characters whose values are correlated in the individuals of a population (FALCONER, 1981). For characters that cannot both be measured on the same individual, there exist no phenotypic correlations, although the characters may be correlated genetically and environmentally. HYDE & EBERT (1978), in order to include such "intersex genetic correlations", redefined "genetic correlation" in their study of aggressiveness in wild house mice as a correlation between relatives. Such was done for dominance ability in males and juvenile and female aggressiveness in females in the present study.

The similarity in the incidence of ripeness during the first four female tests in DH and DL females is consistent with the above results (Table 33). Whereas selection for JAS was accompanied by a change in the relative occurrence of ripe females during the first four female tests (see section 4.3.1.), selection for dominance ability does not result in such a change; valid tests, DH ripe:non-ripe = 67:72 and DL ripe:non-ripe = 30:54, χ^2 test, 2-tailed, $0.05 < p < 0.10$; invalid tests, DH ripe:non-ripe = 42:40 and DL ripe:non-ripe = 44:50, χ^2 test, 2-tailed, $0.50 < p < 0.70$; valid + invalid tests, $0.10 < p < 0.20$.

TABLE 33. Incidence of ripe females during the first four female tests, separated according to valid and invalid tests, in the DH and DL line

	Incidence of ripe ♀♀ in first 4 female tests							
	DH				DL			
Generation	Valid		Invalid		Valid		Invalid	
	n	%	n	%	n	%	n	%
1	23	54.8	13	50.0	17	44.7	12	46.2
2	25	44.6	13	50.0	9	36.0	17	54.8
3	19	46.3	16	53.3	4	19.0	15	40.5
Total	67	48.2	42	51.2	30	35.7	44	46.8

4.3.3.4. Dominance in the juvenile and territorial aggression lines.

Hitherto in this section the effect of selection for dominance ability on the aggressiveness of both sexes in other test situations was considered. Addi-

tional evidence for the conclusions drawn from this can be gained from the reverse comparison, viz. the effect of selection for JAS and TAS on dominance ability. In the third generation of selection a number of males from the juvenile and territorial aggression lines were submitted to dominance tests against males of both dominance lines. In this way the mean dominance ability of the other selection lines can be established relative to those of the high and low dominance lines. It then appears that the mean dominance ability of both the high and low juvenile aggression line in generation 3 is comparable to that of the DH line. That is, against males of the DH line males of the JH and JL line win about half of the contests (55.6% and 56.2% respectively), and against DL males JH males become dominant in 80.0% of the tests and JL males in 87.5% (Fig. 40). The difference in percentage of the tests won against DH and DL males in both cases is consistent with the difference in mean dominance ability between the two dominance lines. The similar mean dominance ability of the JH and JL line confirms the absence of a genetic correlation between dominance ability and JAS (see section 4.3.3.1.).

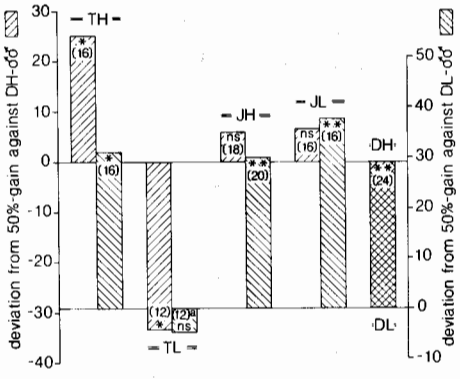

Fig. 40. Correlated responses of dominance ability in generation 3 of the juvenile and territorial aggression lines, expressed as deviations from random (50%-gain) against DH males (left columns, read left scale) and against DL males (right columns, read right scale). The difference between the bases of left and right columns corresponds to the joint response to selection for dominance ability of DH-3 and DL-3 males. Numbers of dominance tests shown in parentheses. Deviations from random tested with χ^2 test, 2-tailed: ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$. [a] One test remained undecided after 40 min and was therefore omitted from the analysis.

Furthermore the lack of response to selection for an enhanced mean dominance ability, as indicated by the test results between C-2 and DH-2 males (see section 3.5.1.), is again convincingly demonstrated.

The territorial aggression lines show a correlated response of dominance ability (Fig. 40). This is unexpected in the light of earlier findings: BAKKER & SEVENSTER (1983) found territorial aggressiveness only of minor importance as a determinant of dominance. Furthermore, in section 4.3.3.1. it appeared that the mean territorial aggression scores of the two dominance lines did not differ significantly. The elevated mean dominance ability of TH males compared to DH males cannot be due to higher level of territorial aggression of TH males, because TH and DH males do not differ in that respect (see secion 4.3.3.1.). BAKKER & SEVENSTER (1983) showed that colouration is an important determinant of dominance in three-spined stickleback males. The colouration of the males used in the inter-line dominance tests was therefore assessed after the tests by stimulating the males with a ripe female. Males of the TH line were the most brightly coloured and TL males the most dull coloured males of the different selection lines in generation 3 (Table 34) (cf. ROWLAND, 1984, who also found a positive correlation between colouration and territorial aggression in the three-spined stickleback). So the correlated response of dominance ability in the territorial aggression lines is no doubt the result of a change in the brightness of colouration in TH and TL males, which is comparable but more pronounced than in males of the dominance lines.

The difference of mean dominance ability between DH-3 an DL-3 males as it was assessed directly (+ 29.2%, that is 79.2%, of the tests won by DH), and as it can be deduced from dominance tests against TH males, that is 31.2% (TH *vs* DL) -25.0%

TABLE 34. Mean colouration scores of selection lines in generation 3, assessed with courtship tests after dominance tests between males of the selection lines and DH or DL males

Dominance tests between	Mean colouration score of DH	DL	Other line	
DH-DL	1.65	2.00	—	
DH-TH	1.56	—	1.31	TH: 1.22
DL-TH	—	1.88	1.13	
DH-TL	1.25	—	2.42	TL: 2.22
DL-TL	—	2.27	2.00	
DH-JH	1.56	—	1.39	JH: 1.42
DL-JH	—	1.80	1.45	
DH-JL	1.50	—	1.56	JL: 1.63
DL-JL	—	1.88	1.69	
Total mean score	DH: 1.52	DL: 1.94		

(TH *vs* DH) = +6.2% of the tests to be won by DH, are dissimilar (see Fig. 40). This may be due to a combination of the following reasons. First, sampling causes deviations from the expected difference, which serves as an estimation of the real difference and is also liable to sampling errors. Even if all TH males had dominated DL males, the deduced difference (50.0-25.0 = +25.0%) between the DH and DL line would stay below the observed difference of +29.2% in mean dominance ability. Secondly, there probably is an effect of the test-sequence of the lines; experience of dominance or inferiority in the previous test is also an important determinant of dominance (BAKKER & SEVENSTER, 1983). The test-sequence in this case was DH/TH, DL/TL first and DH/TL, DL/TH thereafter. The relatively positive experience of DL males against TL males may have caused a temporary increase of the mean dominance ability of DL males, and thus the reduction of the difference in mean dominance ability between the two dominance lines, as concluded from the tests against TH males.

It is interesting to note that while selection for enhanced territorial aggressiveness was unsuccessful such selection does result in increased brightness of colouration (see Table 34; the mean colouration score of DH males is comparable to that of control males, see section 3.5.3.2.). This topic will be discussed further in chapter 5.

4.3.4. *Genetic correlations.*

To supplement the qualitative judgement of the different genetic correlations in the previous three sections, this section presents some quantitative data relevant to this topic. As mentioned in section 4.2.4., the estimates of genetic correlations are subject to large sampling errors. I will therefore present only the most reliable estimates, viz. the genetic correlations of aggression scores obtained from aggression tests on both sexes of the low selection lines. The progressive increase of the cumulative response and correlated response of the JL and TL line during selection are more or less linear. The mean responses and correlated responses per generation are therefore reflected by the regression coefficient of mean aggression score on generations of selection (NAGAI *et al.*, 1978). With the regression coefficients calculated from the data presented in Fig. 29 and 31, the genetic correlations between JAS and TAS in males and between JAS and FAS in females can be estimated according to the formula given in section 4.2.4. (Table 35). The calculated genetic correlations agree well with the genetic correlations obtained qualitatively in section 4.3.1. and 4.3.2. The genetic contribution to variation in aggressiveness of juvenile and adult females is most likely identical. In males the genetic correlation of aggression scores in both developmental stages is far weaker, and because of differences in nest-site choice between JL and C males (see section 4.3.2.) this value is probably even an overestimation. The genetic influence on variation in territorial aggressiveness is at least partly different from that on variation in juvenile aggressiveness in males.

TABLE 35. Genetic correlations (r_a) between juvenile (JAS) and territorial aggression scores (TAS) and between JAS and female aggression scores (FAS) in the low lines

Character selected	R and CR of JAS	CR and R of TAS	CR and R of FAS	Realized[a] r_a
JAS (♂♂)	-4.90	-4.07	—	0.50
TAS	-2.90	-9.81	—	
JAS (♀♀)	-5.27	—	-2.32	0.98
FAS	-5.73	—	-2.64	

R = response (relative to mean score of C-0 and C-2), CR = correlated response (relative to mean score of C-0 and C-2), r_a according to formula in section 4.2.4.

It is interesting to note that the above presented genetic correlations have the same sign as the corresponding phenotypic correlations in the base population and are of similar magnitude (see section 2.5.2.). This is to expected if genetic and environmental sources of variation affect the characters through similar physiological mechanisms (FALCONER, 1981).

4.3.5. *Conclusions.*

If responses and correlated responses of the different criteria of selection are compared, the picture emerges of genetic correlations between aggressiveness in different test situations that is similar to the matrix of phenotypic correlations in the base population (see section 2.5.2.). That is, the genetic influence on variation of aggressiveness in juvenile and adult females is probably identical. In males the situation is more complicated. Aggressiveness of juvenile males is indiscernible from that of juvenile females and variation in juvenile aggressiveness is only partly governed by the same genetic factors as variation in territorial aggressiveness. Juvenile aggressiveness and dominance ability are genetically uncorrelated. Variation in territorial aggressiveness and in dominance ability are probably effected by a different genetic background, since two-way selection for dominance ability does not result in a significant difference between territorial aggressiveness of the high and low line. However, two-way selection for territorial aggressiveness is accompanied by a correlated response of dominance ability which can be traced back to a difference in the brightness of colouration between males of the two territorial aggression lines that is more pronounced than in males of the two dominance lines. Brightness of colouration can apparently be affected through more than one, genetically distinct,

physiological pathway. Thus 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.

4.4. Asymmetrical responses and lack of response.

4.4.1. *Asymmetry of response.*

In general, selection for aggressiveness in upward direction was less effective than in downward direction (JH/JL lines, see section 3.3.1.; TH/TL lines, see section 3.4.1.1.; DH/DL lines, see section 3.5.1.). In the previous chapter some factors were analysed that influence the selection differential and hence the response to selection, such as limitation in spawning (JH line) and reduced embryonic viability (all high lines, but most pronounced in the DH line). If we leave aside the possibility of selective embryonic mortality (but see section 4.4.2.), then these factors cannot explain the lack of response in the TH and DH line, and they can have contributed only to a minor extent to the relapse of response in JH-3.

FALCONER (1981) enumerates no less than seven main causes that, in addition to differences of the selection differential, may generate asymmetrical responses.

Maternal effects (post-natal), or in the case of sticklebacks paternal effects, as a cause for asymmetry have been minimized by removal of the clutches from the nests shortly after fertilization, after which aeration of the eggs occurred artificially.

Indirect selection could lead to asymmetrical responses if the criterion of selection is not quite the same as the character measured for assessing the response and if there exists a non-linear relationship between the two measures. This cause for asymmetrical responses is irrelevant to the present study.

Another cause for asymmetry of response is random drift. In the present study random drift may have influenced the estimates of the different realized heritabilities, for the number of parents in each generation was small, indeed. With replications of selection lines the influence of random drift could have been assessed. The correlated responses of the different criteria of selection partly compensate the need of such replicate selection lines. These correlated responses (see Figs 29, 31, 34 and 40) suggest a limited influence of random drift, for if random drift had caused the lack of response in males of the TH and DH line one should expect from the correlated responses some evidence for enhancement of territorial aggressiveness or dominance ability above the control level. This was obviously not the case. The fluctuating response of the JH line (both sexes) might to some extent be due to random drift.

Another cause for asymmetrical responses is inbreeding depression. The usual way to reveal how much asymmetry can be attributed to this cause is by means of an unselected control population that is subjected to the same degree of inbreeding. In the present study the different selection lines, which were all inbred to a comparable degree, and their correlated responses of the different criteria of selection, served as mutual controls. The C line, which was not inbred to any extent, was maintained to detect environmental influences. Inbreeding depression cannot explain the lack of response in males of the TH and DH line, since the first two generations of the selection lines were only slightly inbred (Table 36). Some inbreeding could no longer be avoided in breeding generation 3, which might explain why many aggression scores are lower in generation 3 than in the preceding generation. Likewise, the decline of mean JAS in JH-3 (both sexes) might partly result from inbreeding.

A sixth cause that may generate asymmetrical responses is scalar asymmetry. This situation occurs if the genetic and environmental variation are skewed to different degrees or in opposite directions. The genetic variation will then make up a larger proportion of the total at one end of the distribution than at the other. The difference in

TABLE 36. Coefficients of inbreeding of the different selection lines during selection

Line	Coefficient of inbreeding in generation			
	0	1	2	3
JH	0.00	0.00	0.09	0.20
JL	0.00	0.00	0.00	0.25
TH	0.00	0.00	0.00	0.14
TL	0.00	0.00	0.03	0.13
DH	0.00	0.00	0.08	0.20
DL	0.00	0.00	0.08	0.12

skewness may be due to genotype-environment interaction. Males with a high level of territorial aggression may, for example, be more susceptible to environmental variation than males with a low level. Males with high scores will then exhibit a lower heritability than those with low scores. This may well have contributed to the lack of response in males of the TH and DH line and, possibly to the negative response in JH-3. A non-linear offspring-parent regression in the base population should be expected from scalar asymmetry, but it is unfortunate that evidence related to scalar asymmetry cannot be obtained from the present data.

The last two main causes for asymmetrical responses are connected with genetic asymmetry. The additive genetic variance and thus heritability depends on gene frequencies. If all genes affecting a character were at the symmetrical gene frequencies in the base population, that is those gene frequencies with which genes contribute maximally to the heritability (for additive genes 0.5 and for recessive genes with a gene frequency of 0.75 of the recessive allele), two-way selection would be expected to yield a symmetrical response, as in the selection for female aggressiveness. Considering the high genetic correlation between female aggressiveness and juvenile aggressiveness, two-way selection for the latter should also yield symmetrical responses. An expectation for the irregularities in the response of the JH line is therefore likely to be found in one or more of the above mentioned causes for asymmetry of response. But if the average gene frequencies in the base population are above or below these symmetrical gene frequencies, then in one line gene frequencies will move away from the symmetrical values and the heritability will diminish with the progress of selection; in the other line heritability on the other hand will increase. This will therefore result in an asymmetrical response. But such asymmetry depends on differentiation in gene frequencies and would therefore not be expected to appear immediately in the first few generations. Furthermore, it would be associated with non-linear responses. Because the lack of responses in males of the TH and DH line clearly fails to meet these expectations, genetic asymmetry as a major cause for the asymmetry of responses has to be rejected in these cases.

A special case of genetic asymmetry is the genetic asymmetry of genes with large effects. In this case the first selection of parents produces a large change of gene frequency, equivalent to many generations of selection on genes with small effects. Asymmetry that results from this cause is immediate. For dominance ability such genes with large effects may be involved, since selection is most effective with parents from the base population (see Table 26). This cause can, however, not explain the lack of response in males of the TH and DH line, no more than the foregoing seven causes.

An analysis of the different, possible causes for asymmetry of response to bidirectional selection (see above) reveals that the decrease of juvenile aggressiveness in JH-3 is probably a result of the combined effect of limitations at breeding (discussed in section 3.3.1.), inbreeding depression of generation 3, and a higher susceptibility to environmental variation of fish with high juvenile aggression scores. Although the latter cause may also be effective in males with a high territorial aggression score or with a high dominance ability, none of the possible causes for an asymmetry of response can satisfactorily explain the total lack of response in the TH and DH line.

4.4.2. *Lack of response.*

For the lack of response in males of the TH and DH line an explanation in terms of the consequences of natural selection seems more plausible, since, after all, the population of sticklebacks from which samples were caught for the present study has been subjected to many generations of such selection. The failure to select for enhanced territorial aggressiveness and enhanced dominance ability could mean that the present study started with a base population already at the upper selection limit for those characters. This natural selection pressure in favour of high aggression phenotypes in males, if such exists, has not lead, however, to fixation of all relevant loci, as demonstrated by the clear response to selection for low aggression phenotypes in males (TL and DL line). The simple picture, that by the time the selection limit is reached all relevant loci have been made homozygous, has experimentally proven to be wrong in many cases (see *e.g.* FALCONER, 1981). Loci with recessive alleles may remain unfixed. Recessive alleles that are unfavourable (in this case: that will contribute to low territorial aggressiveness or to low dominance ability) will be brought to low frequencies. Selection is then very slow to reduce their frequencies further, because most of the variance they contribute is non-additive. The genetic architecture of characters that have been subjected to directional selection in their evolutionary history is characterized by directional dominance (BREESE & MATHER, 1960), in which the influence of the dominant alleles acts predominantly in the direction of selection. In the present study one expects that the alleles that contribute to high territorial aggressiveness and high dominance ability will be dominant.

Thus characters which have been subjected to directional selection are expected to display a high level of dominance variation, relative to the level of additive variation, whereas characters under the influence of stabilising selection will show a large additive genetic component,

relative to the dominance component. A comparison of the proportion of the two main components of the genetic variance of territorial aggressiveness and female aggressiveness reveals that the dominance component clearly takes up a greater part of the genetic variance in the case of territorial aggressiveness (Table 37). So the genetic architecture of territorial aggressiveness of males supports the view that this character has been subjected to directional selection in nature.

TABLE 37. Proportions of additive genetic variance (V_A) and dominance variance (V_D) of territorial aggression score (TAS) and female aggression score (FAS)

Aggression score	t_{FS}	h^2	$V_A : V_D$
TAS	0.29	0.23	0.23 : 0.70
FAS	0.24	0.31	0.31 : 0.34

t_{FS} = full sib correlation in the base population (see Table 14), h^2 = realized heritability estimated from the joint response of the TH and TL line (see Table 22). $V_A : V_D$ estimated according to $h^2 : 2(2t_{FS}-h^2)$.

On theoretical grounds, presented above, it is expected that three-spined stickleback males with a high level of territorial aggression are favoured by natural selection. On the other hand, there are selection pressures against extreme high levels of territorial aggression (see discussion, section 5.2.2.). Is there experimental evidence for higher fitness of this phenotype? The most direct evidence for a relation between territorial aggressiveness and fitness is offered by a laboratory study of VAN DEN ASSEM (1967): in a stabilized situation with rival males territorial aggressiveness (expressed as the number of attacks initiated by the owner) is positively correlated with territory size, which, in turn, is positively correlated with the probability of getting a female in the nest. Moreover, males with large territories suffered less often from egg-stealing by neighbouring males. These findings were confirmed in the laboratory by LI & OWINGS (1978a), who reported that in groups of reproductive males dominant males (in a somewhat different sense than in the present study) maintained larger territories, were more aggressive, fed more, were more successful in nest establishment, and may have been less susceptible to nest raiding, than subordinate males. During courtship dominant males seemed to have priority of access to females. The same authors assessed that the highest status neighbours tended to raid the most and that raiding presumably reduced the raided male's courtship motivation (LI & OWINGS, 1978b).

Another line of evidence that high territorial aggression phenotypes in three-spined stickleback males are favoured is offered by the comparison of the level of territorial aggression between related species. The three-spined stickleback *Gasterosteus aculeatus* possesses long, stout spines, whilst the spines of the nine-spined stickleback *Pungitius pungitius* are shorter and less robust. The three-spined stickleback is therefore better protected against predators such as pike (*Esox*) and perch (*Perca*) (HOOGLAND *et al.*, 1957). Various aspects of the *Gasterosteus* male's behaviour, in comparison to *Pungitius* males, are correlated with this difference in the anti-predator adaptations of the two genera of sticklebacks. WILZ (1971) showed that the *Pungitius* male is far less aggressive than *Gasterosteus*, both in courtship and male-male encounters (assessed with tests comparable to the ones applied in the present study) (see also HUNTINGFORD, 1977). The degree of freedom from predators of the three-spined stickleback is probably a primary factor in permitting, during the course of evolution, the severe competition between males for suitable territories, which is undoubtedly a conspicuous activity. It would therefore be very interesting to subject a population of nine-spined sticklebacks to artificial selection pressures such as in the present study. Territorial aggressiveness of three-spined stickleback males is also greater compared to that of other stickleback species such as the four-spined stickleback, *Apeltes quadracus*, and the black-spotted stickleback, *Gasterosteus wheatlandi* (ROWLAND, 1983a, b).

However, between different populations of the three-spined stickleback the mean level of territorial aggression can also vary considerably (WILZ, 1973; HUNTINGFORD, 1982). To some degree HUNTINGFORD (1982) could relate this interpopulational variation in territorial aggressiveness to the level of predation to which the sticklebacks were exposed. Since in the population sampled for the present study predation by large predatory fish is most likely low (based on the apparent absence of potential fish predators of *G. aculeatus*), it is plausible from the foregoing data to assume that an upper selection limit for territorial aggressiveness has been reached in this population.

The proposed upper selection limit for dominance ability probably has a different nature than that for territorial aggressiveness. Contrary to selection for enhanced territorial aggressiveness, selection for enhanced dominance ability is accompanied by a clear reduction in viability of fertilized eggs (see section 3.5.1.). Both the lack of response and this increased embryonic mortality in the DH line may be explained by a superiority of heterozygote genotypes under the joint action of artificial

and natural selection. The applied artificial selection in the DH line favours males with a high dominance ability. A response to this selection may be counteracted by natural selection, because of reduced viability of offspring with genotypes for "enhanced dominance". This would be the case when genes (perhaps one or a few with large effects) that increase dominance ability cause mortality in homozygous condition, either as a pleiotropic effect of the genes involved, or as an indirect effect because of close linkage with other, lethal genes. In both cases the increasing genes in question will be maintained in a heterozygote condition and as a consequence there is no response to selection and an increased mortality of some of the offspring. However, the nature of the upper selection limit for dominance ability in the three-spined stickleback population of the present study, outlined above, remains hypothetical, although in *Drosophila* populations this seems to be a common situation at the selecion limit (see FALCONER, 1981).

In summary, the most plausible explanation for the total lack of response in the TH and DH line is that the base population has already been at the upper selection limit for territorial aggressiveness and dominance ability. This view is supported by data from the literature. In the case of dominance ability selection in upward direction is accompanied by a severe reduction in viability. The upper selection limit for dominance ability may therefore be interpreted in terms of a superiority of heterozygote genotypes under the combined action of artificial and natural selection.

4.5. Correlated responses of additional measures of aggressiveness.

In section 4.3. it was shown that the genetic causation of variation in intra-specific aggressiveness of males in different contexts is by no means identical. In this section several measures of aggressiveness within a given situation are compared if selection is exerted on one of them (namely on the % biting-bumping time). This is done in order to determine whether or not variation of different manifestations of aggressiveness in a particular situation have the same genetic basis.

The following measures of aggressiveness are considered:

1. % biting-bumping time in the tube-period (or test-period in the case of male tests). This measure served as the criterion of selection and is used here as the frame of reference for the other measures of aggressiveness;

2. meet latency. The time between presentation of the opponent to the subject and the arrival of the latter at the tube. The maximal time possible is 300 sec;
3. attack latency. The time between a subject's first arrival at the tube and its first overt aggression (biting or bumping) directed at the opponent. The maximal time possible is 300 sec;
4. % at tube in the test-period. The time, as a % of the total 300 sec test-period, spent within a fish-length distance of the tube;
5. mean bout length of biting-bumping time. Although single bites or bumps occur, most biting and bumping occurs in bouts of variable duration, during which a number of bites and/or bumps are directed in quick succession at the opponent;
6. number of intense bites. Some bites are distinctly higher in intensity, and thus distinguishable from regular bites. These bites are mostly observed at the end of biting-bumping bouts or as single bites.

The above presented list is not exhaustive, but merely a selection of some obvious measures of aggressiveness used to test the hypothesis of a common genetic basis for variation in different manifestations of intraspecific aggressiveness in a specific context. To this end, the relative incidence of threatening displays would also be of interest here. But unfortunately, under the applied test conditions the opponent practically never shows aggressive behaviour and therefore threatening displays were only occasionally observed.

In the following analysis mean aggression scores of third generation high and low line fish and of C-0 fish are compared pairwise. In the case of "mean bout length of biting-bumping time" a comparison is made with the C-2 level instead of the C-0 level. This was done because in the base population, contrary to later generations, biting-bumping time bout intervals of very short duration (less than about 0.5 sec) were neglected. Although this procedure has little influence on the % biting-bumping time in the tube-period (a maximal shift of only a few percentages can be attributed to this different recording method), the mean bout length of biting-bumping time is more affected. For that reason the C-2 level is taken as a reference point for the mean bout length.

Different measures of juvenile aggressiveness in females are, as in juvenile males (see section 2.1.3.), phenotypically correlated with each other. Selection for % biting-bumping time in the tube-period also results in changes of other measures of juvenile aggressiveness in females, similar to those of the selection criterion. Thus, most changes in the low line reach the level of significance compared to C-0, while the correlated

responses in the high line are, like the response after three generations of selection, nonsignificant (Fig. 41). Variation in each of the different measures of juvenile aggressiveness in juvenile females is therefore most likely influenced by identical genetic variation.

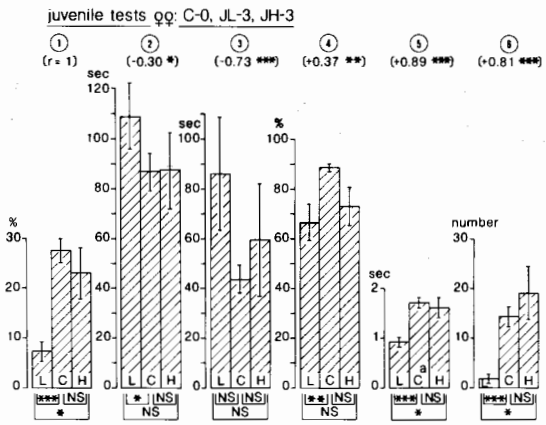

Fig. 41. Juvenile aggressiveness of JH-3 and JL-3 females: responses of the % biting-bumping time in the tube-period (1), and correlated responses of some other measures of aggressiveness (2 = meet latency, 3 = attack latency, 4 = % at tube in test-period, 5 = mean bout length of biting-bumping time, 6 = number of intense bites; generation-means are based on individual means of tests prior to week 15 after fertilization). Values in brackets represent the correlation coefficients of each measure with JAS for C-0 juvenile females (fish with at least two valid juvenile tests) (t test, 2-tailed: * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$). The distributions of JH-3, JL-3 and C-0 ([a]C-2) are compared pairwise with the Mann-Whitney U test, 1-tailed: ns = $p>0.05$, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$. Lengths of bars represent two standard errors of the means.

In juvenile males the situation is comparable (Fig. 42); the phenotypic correlations between % biting-bumping time in the tube-period and other measures of juvenile aggresssiveness are moderate to high (see also section 2.1.3.) and the correlated responses of the various measures of aggressiveness agree well with the responses of the selection criterion. However, due to lower number of tested juvenile males as compared to the females (juvenile males in JL-3: 13 and in JH-3: 11, whereas juvenile females in JH-3: 16 and in JL-3: 15; see Table 15), the 5% level of significance is here not reached for most measures of aggressiveness.

The picture is statistically more convincing in the case of female aggressiveness, because here the sample sizes are reasonably large (TH-3: 16 and TL-3: 14; see Table 21), and selection was equally successful in

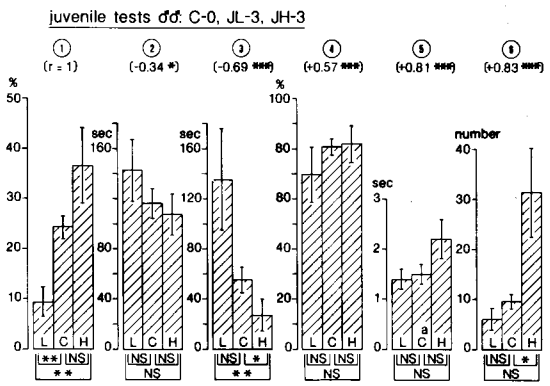

Fig. 42. Juvenile aggressiveness of JH-3 and JL-3 males: responses of the % biting-bumping time in the tube-period (1), and correlated response of some other measures of aggressiveness (2 = meet latency, 3 = attack latency, 4 = % at tube in test-period, 5 = mean bout length of biting-bumping time, 6 = number of intense bites; generation-means are based on individual means of tests prior to week 15 after fertilization). Values in brackets represent the correlation coefficients of each measure with JAS for C-0 juvenile males (fish with at least two valid juvenile tests) (t test, 2-tailed: * = $p < 0.05$, *** = $p < 0.001$). The distributions of JH-3, JL-3 and C-0 ([a]C-2) are compared pairwise with the Mann-Whitney U test, 1-tailed: ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$. Lengths of bars represent two standard errors of the means.

both directions. Most of the measures of female aggressiveness change significantly after three generations of selection for the % biting-bumping time in the tube-period (Fig. 43). However, meet and attack latencies in the low line do not show the expected extension in time. In the high line, on the contrary, selection for a higher FAS is accompanied by a shortening of meet latencies (almost significant at the 5% level compared to C-0 and to TL-3) and even a more pronounced decrease of attack latencies. These asymmetrical results may be ascribed to the fact that in TL-3 many invalid female tests were scored (see section 3.4.1.2.); these were not incorporated in the calculation of the mean meet latency. This exclusion of latencies longer than 300 sec is a plausible reason for the lack of a correlated response of meet latency (and, possibly, also of attack latency) in the low line. If the invalid female tests are counted in as 300 sec scores, then the mean meet latencies are: TL-3 210.4 sec, C-0 114.5 sec and TH-3: 94.7 sec.

Selection for territorial aggressiveness on the basis of the % biting-bumping time in the test-period (which was only successful in the decreased direction) also causes expected changes in other measures of territorial aggressiveness in the TL line (Fig. 44). Because of low

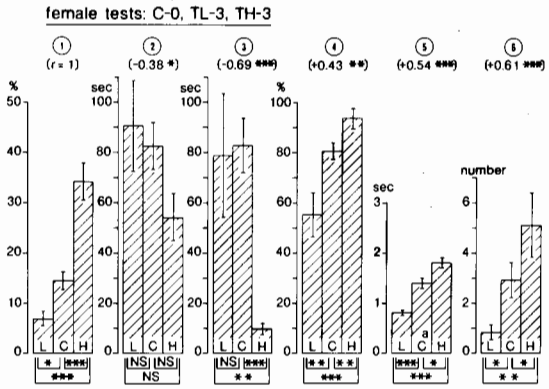

Fig. 43. Female aggressiveness of TH-3 and TL-3 females: responses of the % biting-bumping time in the tube-period (1), and correlated responses of some other measures of aggressiveness (2 = meet latency, 3 = attack latency, 4 = % at tube in test-period, 5 = mean bout length of biting-bumping time, 6 = number of intense bites). Values in brackets represent the correlation coefficients of each measure with FAS for C-0 females (fish with at least two valid female tests) (t test, 2-tailed: * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$). The distributions of TH-3, TL-3 and C-0 ([a]C-2) are compared pairwise with the Mann-Whitney U test, 1-tailed: ns = $p>0.05$, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$. Lengths of bars represent two standard errors of the means.

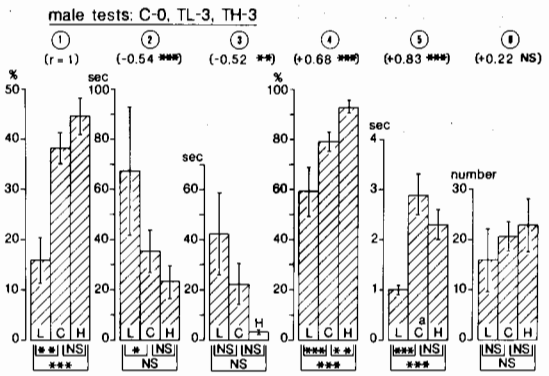

Fig. 44. Territorial aggressiveness of TH-3 and TL-3 males: responses of the % biting-bumping time in the test-period (1), and correlated responses of some other measures of aggressiveness (2 = meet latency, 3 = attack latency, 4 = % at tube in test-period, 5 = mean bout length of biting-bumping time, 6 = number of intense bites). Values in brackets represent the correlation coefficients of each measure with TAS for C-0 males (fish with at least two valid male tests) (t test, 2-tailed: ns = $p>0.05$, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$). The distributions of TH-3, TL-3 and C-0 ([a]C-2) are compared pairwise with the Mann-Whitney U test, 1-tailed: ns = $p>0.05$, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$. Lengths of bars represent two standard errors of the means.

numbers of tested males (TL-3: 7, for reasons see section 3.4.2.) not all measures of aggressiveness change significantly. The data from the TL line seem, nevertheless, to be consistent with the view that within one test situation variation in different measures of aggressiveness are manifestations of the same genetic variation.

However, in the TH line some inconsistencies with this view can be noticed in Fig. 44: although no response to selection for a higher % biting-bumping time in the test-period was achieved, some other measures of territorial aggressiveness do show a change, suggesting an effect of selection, and in the case of % at the tube in the test-period even a highly significant increase relative to the control level is reached (TH-3: 14 males). These changes might be attributed to the simultaneous selection for high female aggressiveness in the TH line, which, in contrast with high territorial aggressiveness in the males, was successful. If this is the case, then apparently not all manifestations of territorial aggressiveness are influenced to the same extent by the female's genetic contribution. This could mean that not all manifestations of territorial aggressiveness are under the same genetic control.

The phenotypic correlation of the % biting-bumping time in the test-period during the male tests and the number of intense bites is unexpectedly low (see Fig. 44), unlike the corresponding coefficients of correlation in the other test situations (see Figs 41, 42, 43). The number of intense bites is apparently not a reliable indicator of the time spent in biting-bumping during the male test. This must probably be ascribed to the fact that in male tests, as compared with juvenile and female tests, more intense bites are single bites and not bites that end biting-bumping bouts. This is especially the case during the first few minutes of the male test-period when there is a preponderance of many single intense bites, which contribute only little to the total time spent in biting-bumping. It is even possible that territorial males that vigorously attack an enclosed intruder with many single intense bites gain relatively low territorial aggression scores. Territorial aggressiveness measured as the total number of bites (see WOOTTON, 1976), or as total duration of biting-bumping used in the present study, disregard the intensity of biting. The total duration of biting-bumping is, however, much better correlated with other measures of territorial aggressiveness (see correlation coefficients in Fig. 44) than the intensity of biting (correlation coefficients of the number of intense bites in C-0 males with meet latency: -0.23, with attack latency: -0.31, with the % at tube in test-period: 0.09, with the mean bout length of biting-bumping time: 0.02; all correlation coefficients do not differ significantly from 0, t test, 2-tailed, df 33). The total duration of biting-bumping is therefore a more adequate measure of the territorial aggressiveness in males.

In summary, this section shows that different measures of juvenile aggressiveness and female aggressiveness are affected in similar ways to selection exerted on one of these measures. Variation in each of the different measures of juvenile and female aggressiveness is therefore most likely influenced by the same genetic variation. In the case of males, however, different manifestations of territorial aggressiveness are possibly not all under the same genetic control.

As mentioned earlier in this section, threatening displays were only occasionally, and then often incomplete or of very short duration, observed in the behavioural tests, except during dominance tests, in which males could interact freely. An impression of the incidence of threatening for fish of the control line (generation 2) is given in Table 38. In female tests threatening was never observed, not in females of the control line, nor

TABLE 38. Incidence of threatening in C-2 fish

Type of test	Total number of tests	Tests positive for threatening		
		Number	%	Mean total duration of threatening (sec)
Juvenile test ♂♂	52	3	5.8	5.6
Juvenile test ♀♀	55	2	3.6	1.2
Male test	67	6	9.0	2.3
Female test	81	0	0.0	—

in the different selection lines. Selection for aggressiveness in the different test situations was not accompanied by clear changes in the incidence of threatening. In all selection lines (generation 3) threatening was observed in only a minor proportion of the tests and in those positive tests the mean total time spent threatening per line did not exceed 8 sec of the 300 sec test-periods (2.7%). Interesting to note is the absence of threatening in juvenile tests of JL-3 and in male tests of TL-3.

A most peculiar behaviour that was observed during the behavioural tests, especially during the male and female tests, is "snapping at air-bubbles", which as far as I know has not been described earlier. In a sexual context air-bubbles act often as stimuli which evoke zigzagging, which is then called "vacuum zigzagging" (*e.g.* SEVENSTER, 1961). But snapping at air-bubbles is predominantly seen in aggressive contexts. It is often displayed on the way to or from the opponent. From some distance the fish then fixates for some time on an air-bubble, which floats on the water-surface. Thereupon it may loose interest but in many cases it swims rapidly in a straight line towards the air-bubble and bumps it with its snout. This may be repeated several times in succession.

During juvenile tests this behaviour is very infrequent and out of 159 third generation juveniles tested it was only observed in 3 of these, each belonging to a different line. During the male and female tests in generation 3 it was more frequent, especially in the low selection lines. Males of TL-3 and, to a lesser extent, TL-3 females, often performed conspicuous snapping at air-bubbles (Table 39). In fact, this behaviour was noticed for the first time in two males of TL-1. However, a satisfactory explanation for this peculiar behaviour cannot presently be offered. The enhanced frequency of snapping at air-bubbles in the low lines, particularly in the TL line, argues against the interpretation that snapping at air-bubbles is redirected aggression, like leaf-biting (described by SEVENSTER, 1968).

TABLE 39. Incidence of snapping at air-bubbles during male and female tests in the third selected generation of the different selection lines

Line	Male tests			Female tests		
	Number of tests[a]	Tests positive for snapping at air-bubbles		Number of tests[a]	Tests positive for snapping at air-bubbles	
		Number	%		Number	%
JH	21	1	4.8	26	2	7.7
JL	17	2	11.8	20	0	0.0
TH	22	1	4.5	35	0	0.0
TL	12	5	41.7	23	6	26.1
DH	22	1	4.5	33	2	6.1
DL	23	4	17.4	23	3	13.0

[a] half of the total male and female tests have been scored for snapping at air-bubbles.

4.6. Correlated responses of aggression in other test situations.

This section deals with two additional situations in which the level of intra-specific aggression was scored. The first situation concerns the aggressiveness of juveniles in groups, which are composed of JH-3, JL-3 or C-2 juveniles (section 4.6.1.). In section 4.6.2. changes of courtship aggressiveness during selection of males of the various selection lines are analysed.

4.6.1. *Aggression in groups of juveniles.*

To investigate the effect of bidirectional selection for JAS on juvenile aggressiveness in a more natural situation, in generation 3 of the juvenile aggression lines groups of juveniles, consisting of full sibs of the individually isolated fish, were scored weekly for their juvenile aggressiveness (expressed as the total number of bites and bumps in the group, called the flash-number; see section 4.2.2.) until sexual maturity.

In groups of the JH line two distinct peaks of aggression can be discerned during the juvenile phase, whereas in the JL line only the first peak is present (Fig. 45; group-size 15 juveniles, and Fig. 46, top graphs; group-size 5 juveniles). With a group-size of 15 peak levels are relatively high, since individual fluctuations in juvenile aggressiveness, if synchronous, reinforce each other in a group. In groups of 5 juveniles this reinforcement-effect is reduced to such proportions that a comparison with the course of mean individual juvenile aggressiveness with age, as scored with the juvenile tests, is feasible. If one takes into account the dif-

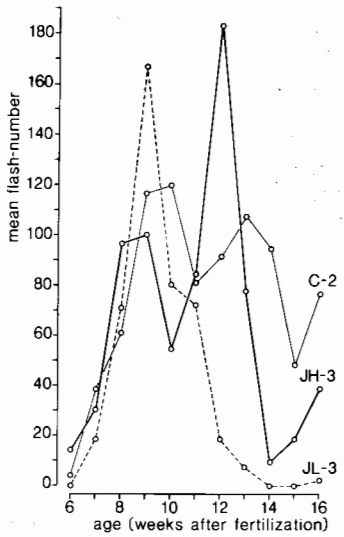

Fig. 45. Mean flash-number as a function of age in groups of 15 juveniles belonging to JH-3 (2 groups), JL-3 (3 groups) and C-2 (5 groups).

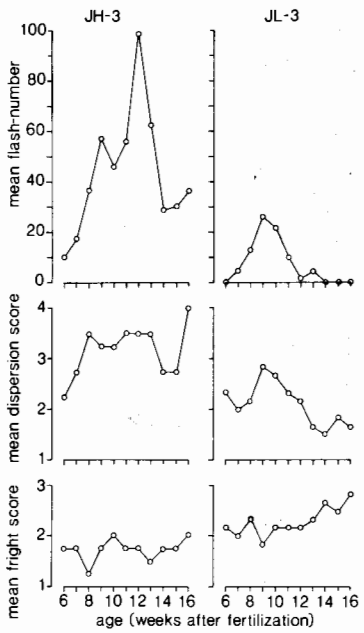

Fig. 46. Mean flash-number, mean dispersion score and mean fright score as a function of age in groups of 5 juveniles belonging to JH-3 (4 groups) and JL-3 (6 groups).

ference in test-frequency (groups were scored weekly, whereas juvenile tests were conducted at two week intervals), the course of juvenile aggressiveness with age is essentially the same in groups and in individuals (compare Fig. 46 top graphs with generation 3 in Fig. 14 and 15, section 3.3.3.). So the juvenile tests are not as artificial as they look at first sight; they measure variations which are also observed in more natural groups of juveniles.

The existence of just one peak of aggression in juveniles of the JL line, as opposed to the two peaks in high line juveniles, suggests a new interpretation of the course of juvenile aggressiveness with age in the base population (see Fig. 9 in section 2.5.1.). Since the base population consists of individuals whose juvenile aggression profiles range from JH to JL, then it is evident that averaging the various juvenile aggression profiles per age-week results in an one-peak course of juvenile aggression with age (the same phenomenon can be seen in the mean flash-numbers as a function of age in C-2 groups; see Fig. 45).

In a functional sense juvenile aggression of the three-spined stickleback is viewed as a means to dispersion (*e.g.* WAI & HOAR, 1963; GOYENS & SEVENSTER, 1976). In the present study the dispersion of the young in groups during their flash-number counts was compared by a four-point dispersion scale (see section 4.2.2.). In groups of 5 juveniles mean dispersion score fluctuates about synchronously with mean flash-number for both the JH-3 and JL-3 groups (compare middle and top graphs in Fig. 46): the higher the mean flash-number the more juveniles scatter. Thus aggression of juveniles in a group leads to their dispersion. The reverse possibility (that fluctuations in mean flash-number are the consequence of changing degrees of scattering) is ruled out, both by these synchronous fluctuations and by the similarity between the juvenile aggression profiles in grouped and isolated juveniles (which means that the changing levels of juvenile aggressiveness must be due to intrinsic causes). The positive correlation between mean flash-number and mean dispersion score also means that juveniles of the JL line move on the average more in a school than JH juveniles (compare middle graphs in Fig. 46; see also Table 40, middle column).

In studying aggression one is always uncertain whether the aggressive response that is measured is influenced by intervening variables such as fear. It is conceivable that the level of aggression is partly a reflection of the degree of fearfulness. In an attempt to disentangle the effects of fearfulness and aggressiveness the reaction of the groups of juveniles to a frightening stimulus was measured on a three-point fright scale (see section 4.2.2.) after their flash-number counts were taken. It appears that mean flash-number and mean fright score are negatively correlated; the

TABLE 40. Mean flash-number, mean dispersion score and mean fright score of JH-3, JL-3 and C-2 juveniles (6th up to and including the 16th week after fertilization)

Line	Group size	Number of groups	Flash-number mean ± S.E.	Dispersion score mean ± S.E.	Fright score mean ± S.E.
JH-3	15	2	65.0 ± 17.0	2.68 ± 0.23	1.60 ± 0.04
JH-3	5	4	44.1 ± 3.1	3.18 ± 0.13	1.73 ± 0.10
JL-3	15	3	40.1 ± 3.0	2.18 ± 0.10	1.97 ± 0.20
JL-3	5	6	7.4 ± 2.0	2.11 ± 0.08	2.29 ± 0.10
C-2	15	5	73.1 ± 24.5	2.38 ± 0.24	1.49 ± 0.22

JH-3 *vs* JL-3 (group size = 5), Mann-Whitney U test, 2-tailed: mean flash-number $p = 0.01$, mean dispersion score $p = 0.01$, mean fright score $p = 0.02$.

lower the level of aggression in the group the stronger the reaction to a frightening stimulus. This is reflected both in the inversely synchronous fluctuations in mean flash-number and mean fright score (cf. upper and lower graphs in Fig. 46) and in the difference of mean fright score between groups of the JH and JL line (Fig. 46 left *vs* right lower graphs, and Table 40 right column). Only in JL groups (15 juveniles) do mean fright scores and mean flash-numbers deviate from those of the C groups (Table 40). This is in accordance with the results of selection for juvenile aggression (see section 3.3.1.).

With the data presented above we cannot separate aggressiveness and fearfulness. The degree of fearfulness may therefore determine the level of aggression in this test situation (cf. HUNTINGFORD, 1976a, b). One might suppose that differences in fearfulness evoked in this situation correspond to differences in fearfulness in other situations. Thus selection for aggressiveness might act on the degree of fearfulness, which in its turn affects the aggressive response. On the other hand it is equally possible that variation in aggressiveness and fearfulness is influenced by common genetic factors, in which case selection for aggressiveness also affects the degree of fearfulness. The present data are insufficient to discriminate between these different possibilities, but the topic is worthy of further study.

The last topic of this section concerns the age of onset of juvenile aggression. The measurements of aggression on individually isolated juveniles and on groups of juveniles started at too advanced an age to establish the age of onset of aggression. Extrapolation of the courses of juvenile aggression as a function of age in the juvenile aggression lines

(Fig. 14 and 15 in section 3.3.3. and Fig. 45 and 46 in this section) suggests that the onset of aggression is earlier in JH juveniles than in those of the JL line. For the precise establishment of the age of onset of juvenile aggression the JH-3 and JL-3 groups were observed daily for the first signs of overt aggression before the flash-number counts. In JH-3 groups aggression is first observed on the average a week earlier than in groups of JL-3 juveniles (Table 41). Precise data for the C line are lacking, but according to the upper limit of the age of onset of aggression in C-2 groups, it is very likely that the JL line deviates more from the C line than the JH line.

TABLE 41. Age at onset of aggression (in days after fertilization) in groups of juveniles belonging to JH-3, JL-3 and C-2

Line	Group size	Age at onset of aggression	Line-mean ± S.E.
JH-3	5	33	JH-3: 34.7 ± 0.8
	5	33	
	5	34	
	15	34	
	15	37	
	5	37	
JL-3	5	38	JL-3: 41.8 ± 1.1
	5	39	
	5	39	
	5	41	
	15	41	
	15	42	
	5	42	
	15	45	
	5	49	
C-2	15	≤35	C-2: 38.0 ± 1.7 (upper limit)
	15	≤35	
	15	36	
	15	>35, ≤42	
	15	>35, ≤42	

JH-3 *vs* JL-3, Mann-Whitney U test, 2-tailed, $p<0.002$.

4.6.2. *Courtship aggression.*

4.6.2.1. Courtship aggression score.

Males of the different selection lines were also scored for courtship aggressiveness throughout the selection experiments by means of courtship tests, in the manner described in chapter 2 for the base population. These correlated responses of courtship aggression are presented in Fig. 47

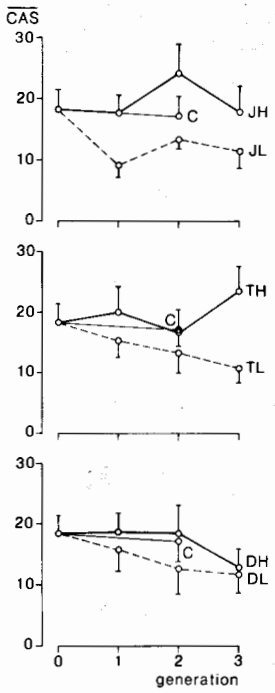

Fig. 47. Correlated responses of courtship aggression score (CAS). Lengths of bars represent one standard error of the mean.

(means and standard errors) and Fig. 48 (frequency distributions). Comparing the graphs of Fig. 47 with those of TAS in the corresponding selection lines (Fig. 31 in section 4.3.2. for the juvenile and territorial aggression lines and Fig. 34 in section 4.3.3.1. for the dominance lines) reveals a similar course of both mean aggression scores with the progress of selection. Like for territorial aggressiveness, for courtship aggressiveness the only significant difference in generation 3 is between males of the two territorial aggression lines (TH-3 *vs* TL-3; Mann-Whitney U test, 1-tailed, $p<0.05$). These results suggest common genetic influences on variation in territorial and courtship aggressiveness. This is in concordance with the generally accepted view that in male three-spined sticklebacks aggression directed against both rivals and ripe females are manifestations of the same motivation, a view which is based on the covarying of territorial and courtship aggression (*e.g.* SEVENSTER, 1961; VAN DEN ASSEM & VAN DER MOLEN, 1969; WILZ, 1973).

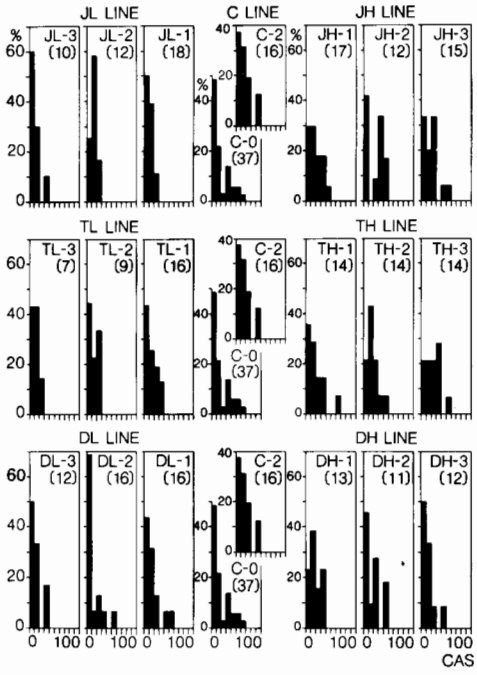

Fig. 48. Frequency distributions of courtship aggression score (CAS) in the different selection lines and in the C line. Numbers of individuals tested in parentheses.

There are, however, some discrepancies between the two test situations. First, in contrast to territorial aggressiveness courtship aggressiveness shows an increase above the control level (TH-3 *vs* C-0; Mann-Whitney U test, 1-tailed, $p < 0.05$). Secondly, the difference observed in territorial aggressiveness between C-0 and C-2 males (see Fig. 31) was not found in courtship aggressiveness. This finding supports the interpretation that a difference in territorial aggression results from a difference in nest-site choice between C-0 and C-2 males (see section 3.4.1.1.), because in courtship tests the test-tube is offered inside the tank and thus the distance between the opponent and the nest is less dependent on the nest-location.

We have seen that direct selection for territorial aggressiveness results in indirect selection for courtship aggressiveness, as is also apparent from the correlated selection differentials on courtship aggressiveness in the territorial aggression lines (Table 42). Without knowing the genetic correlation between TAS and CAS, it is, however, not possible to use this correlated selection differential to estimate the heritability of CAS (FALCONER, 1981).

4.6.2.2. Additional measures of courtship aggressiveness.

The indirect selection for courtship aggressiveness in the territorial aggression lines has also brought about correlated responses of measures for courtship aggressiveness other than CAS. The general picture that

TABLE 42. Results of indirect selection for courtship aggressiveness in the TH and TL line

Genera- tion	TH Selection Line Tested ♂♂ n	$\overline{CAS}$	Selected ♂♂[a] n	$\overline{CAS}$	Cumulative CR	Cumulative weighted CS	C Line Tested ♂♂ n	$\overline{CAS}$
I	37	18.3	3	33.0	0.0	0.0	37	18.3
II	14	19.8	2	19.9	+2.1	+16.9	—	—
III	14	17.0	3	21.4	-0.7	+23.5	16	17.1
IV	14	23.7	2	4.6	+6.0	+30.6	—	—
				TL Selection Line				
I	37	18.3	4	8.9	0.0	0.0		
II	16	15.3	2	13.2	-2.4	- 8.0		
III	9	13.5	2	8.8	-4.2	- 9.7		
IV	7	10.8	1	1.5	-6.9	-13.5		

$\overline{CAS}$ = mean courtship aggression score, CR = correlated response in regard to mean of C-0 and C-2, CS = correlated selection differential, n = number of fish, [a] ♂♂ selected according to $\overline{CAS}$.

emerges is in agreement with that for aggressiveness in the other contexts; that is, different measures of courtship aggressiveness show a correlated response in the same direction as the response to (in this case indirect) selection for % biting-bumping time during courtship tests (Fig. 49). There are, however, some discrepancies with aggressiveness in the other test situations (see below), which probably can be ascribed to the interaction of aggressive and sexual tendencies when a sexual stimulus is presented.

Meet latency and relative duration of biting-bumping are weakly correlated in the different tests on C-0 fish (see section 4.5.). This is not surprising, since meet latency is not only influenced by aggressiveness, but also by a number of other variables, such as the position of the fish in the tank, the degree of disturbance, and the level of fearfulness. In courtship tests on C-0 males the correlation is even completely lacking (Fig. 49). It must be remembered (see section 2.1.3.) that in both male and courtship tests the test-period started with the first arrival of the experimental male at the tube or, if the male approached with zigzagging, after the first zigzag-bout. Males with a high sexual tendency and males with a high aggressive tendency are likely to approach a ripe female equally soon, such that meet latency and courtship aggression are uncorrelated. Despite this, TL-3 males still show a correlated response of meet

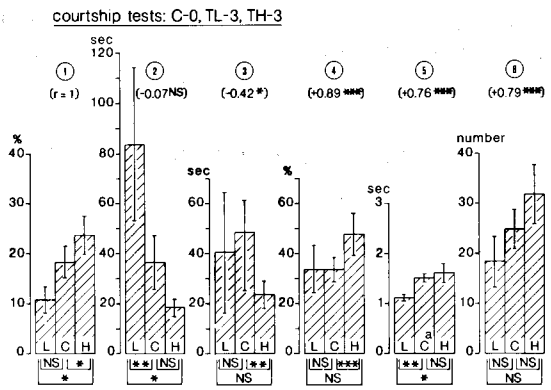

Fig. 49. Courtship aggressiveness of TH-3 and TL-3 males: correlated responses of some measures of aggressiveness (1 = % biting-bumping time in test-period, 2 = meet latency, 3 = attack latency, 4 = % at tube in test-period, 5 = mean bout length of biting-bumping time, 6 = number of intense bites). Values in brackets represent the correlation coefficients of each measure with CAS for C-0 males (fish with at least two valid courtship tests) (t test, 2-tailed: ns = $p > 0.05$, * = $p < 0.05$, *** = $p < 0.001$). The distributions of TH-3, TL-3 and C-0 (^{a}C-2) are compared pairwise with the Mann-Whitney U test, 1-tailed: ns = $p > 0.05$, * = $p > 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Lengths of bars represent two standard errors of the means.

latency in courtship tests. This might mean that both their aggressive and sexual tendencies are reduced. The lack of a correlated response of % at tube in courtship tests on TL-3 males (see Fig. 49) also points to a reduction of the sexual tendency, for a reduced aggressive tendency would allow fuller expression of a sexual tendency (*e.g.* SEVENSTER, 1961) and the concomitant increase in nest-directed activities.

The intensity of courtship aggression is comparable to that of territorial aggression (cf. Figs 44 and 49). The control level of the number of intense bites in these situations is, however, more than twice that of juvenile tests (cf. Fig. 42). The bout length of biting-bumping time in courtship tests (Fig. 49) is, on the contrary, about half that of male tests (Fig. 44), but comparable to bout lengths of overt aggression in juvenile males (Fig. 42). It is likely that the bout length of courtship aggression, compared to that of territorial aggression, is reduced by the appropriate head-up posture of the female (evidence for this was obtained by ROWLAND & SEVENSTER, 1985, by means of dummy experiments). The prolonged biting-bumping bouts observed in male tests are therefore less likely to occur in courtship tests, but the intensity of the attacks in the latter tests is not reduced and is probably inherent in territorial males. The correlation between intensity and duration of aggression, which was low in male tests, is as high in courtship tests as in the other test situations.

In conclusion, it is more difficult to judge whether each of the different manifestations of courtship aggressiveness observed during presentation of a sexual stimulus reflects the same underlying motivation, since one

must consider the concomitant changes in sexual tendency. Furthermore, different measures of courtship aggressiveness are probably influenced to various degrees by the sexual tendency. If the above considerations are taken into account it seems plausible that variation in each of the different measures of courtship aggressiveness is influenced by the same genetic variation.

4.7. Hormonal changes.

Much attention has been paid to the endocrinology of aggressive behaviour in three-spined sticklebacks, particularly in males just before and during the sexual phase. Several studies (see WOOTTON, 1976, and section 5.3.) have shown that before sexual maturity the level of aggression is associated with the level of gonadotropin (a pituitary hormone, the secretion of which is triggered by light). Gonadotropin in turn affects the level of gonadal hormones. These androgens control the male's level of aggression during the sexual phase, whether in combination with gonadotropic hormones or not (see discussion in section 5.3.).

In view of this influence of gonadotropin and/or androgens on the level of aggression before and during the sexual phase, one assumes that the selection experiments of the present study have, in one way or another, acted on the endocrine system. In order to characterize the level of androgens in reproductive males of the different selection lines, the size of their kidneys and their testes, as target organs and endocrine glands of androgens, respectively, were measured in the fourth selected generation.

In male three-spined sticklebacks the kidneys have been transformed from excretory into predominantly secretory organs that produce large amounts of mucus for nest building. These organs, which are small and dark-red in immature males and females, change into strongly swollen, palish pink organs in reproductive males. Their circumference increases up to about seven-fold, which reflects drastic, androgen-dependent changes in the renal tubular cells (WAI & HOAR, 1963; MOURIER, 1972). Recently it was shown that this glandular transformation is effected directly by androgens (DE RUITER & MEIN, 1982).

A comparison of mean kidney size index (see section 4.2.3.) of the different selection lines and of the control line in the fourth selected generation reveals that only territorial aggression line males possess a mean kidney size index deviating from that of C-4 (Table 43: means and standard errors, Fig. 50: frequency distributions). Selection for ter-

TABLE 43. Mean kidney size index of males in the fourth selected generation of the different lines

Line	Number of males	Mean length ± S.E.	Kidney size index mean ± S.E.		
C	39	4.6 ± 0.3	1.50 ± 0.04		
JH	14	4.4 ± 0.3	1.35 ± 0.12	ns	ns
JL	11	4.5 ± 0.1	1.42 ± 0.08	ns	
TH	20	4.8 ± 0.3	1.81 ± 0.09	**	***
TL	31	4.6 ± 0.3	1.26 ± 0.05	**	
DH	4	4.3 ± 0.2	1.53 ± 0.10	ns	ns
DL	15	4.5 ± 0.3	1.43 ± 0.05	ns	

Mann-Whitney U test, 2-tailed, H *vs* C, L *vs* C, and H *vs* L: ns = $p > 0.10$, ** = $p < 0.01$, *** = $p < 0.001$.

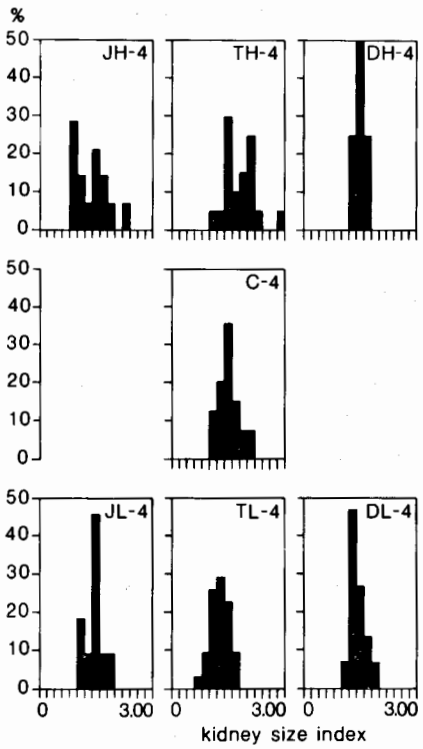

Fig. 50. Frequency distributions of kidney size indices of males in the fourth selected generation of the different lines.

ritorial aggressiveness has therefore most likely resulted in an enhanced level of androgens in TH males and a reduced level in TL males, either directly or indirectly via the level of gonadotropins. A changed sensitivity of the kidney tissues to androgens seems less likely, since selection for territorial aggressiveness is also accompanied by a change in the brightness of colouration (see section 4.3.3.4.).

Since no difference is found in mean kidney size index between JH-4, JL-4 and C-4 males, selection for juvenile aggressiveness has most likely not affected the level of androgens (Table 43 and Fig. 50). The changes in mean age of sexual maturity (see section 3.3.3.) and in the incidence of ripeness (see section 4.3.1.) that accompany selection for juvenile aggressiveness suggest that here selection has acted on the level of gonadotropins.

No change is found in mean kidney size index of DH-4 and DL-4 males relative to C-4 males (Table 43 and Fig. 50), which fits in with the conclusion that dominance ability and territorial aggressiveness are genetically uncorrelated (see section 4.3.3.). However, the number of DH males available for kidney size measurements was small due to the strongly reduced viability in the DH line (see section 3.5.1.) and therefore any conclusions drawn from these DH-4 data must presently be considered tentative.

In addition to the kidney size measurements, the size of the testes of the same males was assessed. A comparison is made between mean testis size indices (see section 4.2.3.) of the different selection lines in Table 44.

TABLE 44. Mean testis size index of isolated and non-isolated males in the fourth selected generation of the different lines

	Isolated males				Non-isolated males			
Line	Number of males	Mean length ± S.E.	Testis size index mean ± S.E.		Number of males	Mean length ± S.E.	Testis size index mean ± S.E.	
	21	4.5 ± 0.1	8.61 ± 0.99		18	4.6 ± 0.1	6.02 ± 0.49	
H	10	4.3 ± 0.1	7.14 ± 1.80	ns	4	4.5 ± 0.1	5.68 ± 1.69	ns
L	3	4.4 ± 0.2	6.48 ± 1.06	ns ns	8	4.5 ± 0.1	3.33 ± 0.31	** ns
TH	13	4.8 ± 0.1	11.37 ± 2.09	ns **	7	4.8 ± 0.1	9.43 ± 1.72	ns *
TL	18	4.6 ± 0.1	4.79 ± 0.32	***	13	4.6 ± 0.1	5.36 ± 0.50	ns
H	4	4.3 ± 0.1	5.62 ± 0.85	ns *	0	—	—	
L	13	4.6 ± 0.1	9.64 ± 0.98	ns	2	4.3 ± 0.1	11.12 ± 5.94	ns

Mann-Whitney U test, 2-tailed, H *vs* C, L *vs* C, and H *vs* L: ns = $p > 0.10$, * = $p < 0.05$, ** = $p < 0.002$, *** = $p < 0.001$.

Despite the larger mean testis size index of isolated males in most selection lines, isolated and non-isolated males are generally comparable. Males of the TH line tend to have enlarged testes as compared to controls, while in TL males the reverse is true. Taken into account the similar changes in kidney size of those males, this difference in testis size between TH-4 and TL-4 males may reflect a difference in the proportion of interstitial tissue, and thus in glandular activity. Males of the JL line tend to possess small testes, but this reduction in mean testis size may, in part, be due to a deformation of the testes; about half of the investigated JL males possessed testes in which the anterior end was bent backward, which is likely to cause an underestimation of the testis-length. Finally, DH-4 males have smaller testes than DL-4 males, which may be related to the decrease in viability found in DH line embryos.

In view of the testis' dual function, interpretation of testis size differences remains uncertain without histological examination. Only tentative suggestions can be made.

4.8. Summary.

In this chapter a number of correlated responses in the various selection lines are analysed.

Comparison of the responses and correlated responses of the different criteria of selection reveals that the genetic correlations between aggressiveness in different test situations are comparable with the phenotypic correlations in fish of the base population. That is, for females the genetic contribution to variation of aggressiveness in the juvenile and adult stage is most likely identical, while in males variation of juvenile and territorial aggressiveness is only partly governed by the same genetic factors. Dominance ability does probably not share genetic factors with juvenile or territorial aggressiveness.

Further analysis of the causes for asymmetrical responses leads to the conclusion, that the lack of response in males to selection for territorial aggressiveness and dominance ability in upward directions is probably due to an upper selection limit for territorial aggressiveness and dominance ability, that is already present in the base population.

Measures of aggressiveness other than the duration of biting-bumping (criterion of selection), such as meet latency, attack latency, % at tube in test-period, mean bout length of biting-bumping time, and number of intense bites, vary in a coherent manner in each of the various test situations. Within each situation variation of these measures is therefore considered to be a reflection of the same genetic variation. However, with respect to territorial aggressiveness some exceptions are found.

The course of juvenile aggression as a function of age in groups of fixed size, composed of high or low juvenile aggression line juveniles (generation 3), is comparable to that of their individually isolated full sibs. Two distinct peaks of aggression appear in high line groups, but only one in low line groups. Juvenile aggression was first observed on the average week earlier in high line groups than in low line groups. Fluctuations in the degree of dispersion and in the degree of fearfulness to a frightening stimulus parallel those of their aggression, as determined by flash-number.

Changes of courtship aggressiveness parallel those of territorial aggressiveness in the various selection lines, suggesting that common genetic influences underlie variation of both manifestations of intra-specific aggressiveness in reproductive males.

Measurements of the kidneys (as indicators of the level of androgens) of reproductive males of the various selection lines (generation 4) show that only males of the territorial aggression lines are deviating from control males: high line males possess enlarged, low line males reduced kidneys. It is argued that selection for territorial aggressiveness has most likely acted on the level of androgens. The changes in testis size are consistent with this view. Correlated responses in the juvenile aggression lines (viz. a difference between high and low line fish in age at sexual maturity, in age at onset of juvenile aggression, and in incidence of ripeness) suggest that selection for juvenile aggressiveness has acted on the level of gonadotropins.

5. General discussion

5.1. Variation in nature and in the laboratory.

One of the issues revealed by this study is the huge variation of intraspecific aggressiveness in the base population in each of the different test situations, even under the standardized experimental conditions (chapter 2). Several kinds of evidence obtained in this study reveal that a considerable part of this variation of aggressiveness is attributable to genotypic variation (chapter 2 and 3). Under natural circumstances, where more environmental variation is present than under the controlled laboratory conditions, one expects that a larger part of the total phenotypic variation is due to environmental variation. It is often argued that the environmental variation of quantitative traits will completely mask the genotypic variation, with the result that under natural circumstances genetic differences only contribute to a negligible extent to the phenotypic variation. With respect to sociobiology this idea gives cause for recurrent scepticism, as mentioned in the introduction of this paper, especially when sociobiological theories are applied to human beings (see *e.g.* RUSE, 1979).

To determine under more variable environmental conditions the importance of genetic differnces that are assessed under standardized circumstances, one should make heritability-estimates under natural circumstances. In view of the, often even insurmountable, difficulties such attempts are hardly made. One of the few investigations of this kind concerns the natural variation in clutch size of the great tit (*Parus major*), based on abundant data from a long-term study of Dutch populations (VAN NOORDWIJK *et al.*, 1981). This analysis has revealed that 40% of the total variation in clutch size under the prevailing natural circumstances is attributable to genetic variation. In the case of aggressiveness of three-spined sticklebacks such a study would be extremely difficult. The greatest obstacles are the individual recognition and the ascertainment of familial relations.

It is evident that under natural circumstances more environmental variables, that are potentially able to influence aggressiveness, are present than under the applied, standardized experimental conditions. In the laboratory variation in abiotic factors is minimized, and *e.g.* predation and intra-specific competition cannot exert their influence on variation of aggressiveness. Without further research it is, of course, impossible to judge whether the degree to which variation of aggressiveness in nature is attributable to genotypic variation would be much lower than under the constant laboratory conditions. But, it is certainly not impossible that even under natural circumstances a high proportion of variation will remain attributable to genotypic variation. In this context, the remarkable similarity between the courses of juvenile aggressiveness as a function of age for isolated juveniles and for their full sibs in a group, may be mentioned. The non-isolated condition, representing a variable present in nature, does apparently not lead to a disappearance of the influence of genotypic variation. The difference in mean juvenile aggressiveness between the high and low juvenile aggression line is as well demonstrable in isolated juveniles as in juveniles in a group (see section 4.6.1.).

5.2. Selection pressures that act on aggressiveness.

Natural selection pressures are often less intense than those that are realized in most artificial selections. To obtain comparable results this, combined with a larger environmental variation, makes natural selection a more time-consuming process than artificial selection in the laboratory, but in essence similar shifts in the levels of aggression can be expected if natural selection consistently favours certain phenotypes during many generations. Artificial enhancement of the reproductive success of phenotypes with extreme aggression levels by directional selection experiments may help to reveal some of the selection pressures which in nature may play a role in keeping aggressiveness within certain limits. In the following three sections selection pressures are discussed that act on dominance ability (section 5.2.1.), on territorial aggressiveness (section 5.2.2.), and on female and juvenile aggressiveness (section 5.2.3.).

5.2.1. *Dominance ability in males.*

Despite consistent selection pressure favouring males with great dominance abilities, the mean level of dominance ability remained unchanged. The facts given in section 3.5.1. suggest that this lack of response might be caused by a reduced embryonic viability of "highly

dominant'' genotypes (see section 4.4.2.). Whether this would happen in nature as well is questionable, since it is not clear whether the reduced viability of progeny in the DH line is a direct consequence of the extreme artificial selection pressures applied. It is conceivable that mortality of ''highly dominant'' genotypes is caused by a close linkage with lethal genes and that genes for enhanced dominance are rarely transmitted without them. The failure of selection for long attack latencies in wild house mice (VAN OORTMERSSEN & BAKKER, 1981), reported in section 3.6., might be ascribed to such linkage with lethal factors. After several unsuccessful attempts to select for longer attack latencies (lines died out immediately or within two generations) a recent attempt appears successful (VAN OORTMERSSEN, pers. comm.).

It must be realized that dominance ability in the present study is a trait measured in an artificial situation. It represents a male's ability to obtain an unoccupied area in competition with another male. How this dominance ability would turn out in more natural situations remains to be seen and is an issue for further research. Assuming that it will manifest itself in a similar way when two males arrive at the same time in a suitable potential territory, several selective forces may keep dominance ability within certain limits in nature, besides the genetic restrictions discussed above.

Brightness of colouration is an important determinant of dominance in three-spined stickleback males in our test situation (BAKKER & SEVENSTER, 1983; see also section 3.5.3.2.) and thus one expects that selective forces with respect to dominance ability act on brightness of colouration. Indications concerning the kinds of selection pressures acting on colouration come from studies on several stickleback populations from the Pacific northwest, which are unusual with respect to the males' nuptial colouration. MCPHAIL (1969) studied populations in which the males became jet black during the breeding season. These ''black'' populations were usually allopatric to normal ''red'' populations (males with bright red throat and blue eyes) and it was shown that the differences in nuptial colours are genetically based (see also HAGEN & MOODIE, 1979). In mate preference tests females of both the ''black'' and ''red'' populations preferred the red male. On the other hand MCPHAIL (1969) suggests that the ''red'' populations suffered more from predation by the western mudminnow (*Novumbra hubbsi*), which eats the eggs and young of the stickleback under laboratory conditions. Experiments show that the mudminnow is attracted more to the red male than to the black male and therefore more likely to find the nest of the former. However, there is no

evidence that *Novumbra* naturally preys on the fry of sticklebacks (McPHAIL, 1969; HAGEN *et al.*, 1980). HAGEN *et al.* (1980) continued to study these black and red populations and convincingly demonstrate that *Novumbra* in a choice test is not orientating to red males because they are more conspicuous, but rather avoids the black. These authors also showed that black sticklebacks are reproductively more successful than reds when competing with *Novumbra* for territories. They suggest that black intimidates *Novumbra*, which is also black, about the size of a stickleback and territorial. In these populations brightness of colouration is counteracted by a selection pressure resulting from inter-specific competition for territories.

Additional selection pressures that act on brightness of colouration are indicated by SEMLER (1971), who studied a population polymorphic for male breeding colours. Only about 14% of the males develop the typical red throat during the breeding season. The others lack red ventral colouration and are instead coloured silver, mottled black, or black. Females from this population prefer to mate with red males, even non-red males whose ventral surfaces are painted red. In addition, red males may be more successful at guarding their nests from the raids of other males. On the other hand, red males are more conspicuous to the rainbow trout which prey heavily on this stickleback population. Another population, in which the typical red breeding colouration was absent in about 80% of the males, was studied by MOODIE (1972a, b). Here it was also experimentally proven that the red males were more subject to predation by the cutthroat trout than were drab males. So in these cases selection through differential predation acts most likely against colouration on the basis of conspicuousness of red males.

The above studies also yield some indications for an advantage of red males in dominance fights. SEMLER (1971) reports an observation made by HAGEN: when red and non-red males are allowed to nest in the same tank, it is usually the red male who acquires the larger territory. Similar results were obtained by HAGEN *et al.* (1980): red males hold larger territories and suffer fewer intrusions than blacks. They suggest that red is a more effective threat than is black. However, without data on territorial aggressiveness of the colour morphs it is uncertain whether these differences in territory size must be solely attributed to colouration differences.

In conclusion, colouration of reproductive three-spined stickleback males is subjected to several selective forces. First, colouration is subject to sexual selection. Females are more attracted to red coloured males

(and probably so much the more to brightly coloured red males). Secondly, red colouration has an intimidating effect on rivals, as already suggested by TER PELKWIJK & TINBERGEN (1937) and confirmed by ROWLAND (1982) and BAKKER & SEVENSTER (1983). Red males are therefore more successful (and brightly coloured red males even more so) in claiming an unoccupied area to settle a territory. But these advantages are balanced by the greater conspicuousness of brightly coloured males to some predators. In populations where predation pressure is low brightly coloured red males would be favoured by natural selection, and as a result an upper limit for dominance ability may be reached. In such populations the reduced viability of offspring of highly dominant males, if occurring in nature, could fix a limit to further enhancement of brightness of colouration.

Selection for territorial aggressiveness was accompanied by a change in brightness of colouration (section 4.3.3.4.). Like DL males, TL males were duller than males in the C line. In contrast to DH males, which were as bright as C males, TH males showed an enhanced brightness of colouration (though no increased territorial aggressiveness). Since nuptial colouration of the three-spined stickleback male is an androgen-dependent secondary sexual character (*e.g.* IKEDA, 1933; HOAR, 1962a, b; BAGGERMAN, 1966), the changes in brightness of colouration in the TH and TL line are in concordance with the difference in kidney and testis size between TH and TL males (section 4.7.), and are most likely caused by concomitant differences in androgen-levels. However, the dull colouration of DL males is probably due to other causes, since indirect measurements of the levels of androgens failed to reveal a difference between DL and C males (section 4.7.). So, causation of differences in brightness of colouration is complex (several factors may be involved, which influence the concentration and/or distribution of black and/or other pigments), and identical deviations, such as the dull appearance of TL and DL males, may have a different causation.

5.2.2. *Territorial aggressiveness in males.*

With respect to territorial aggressiveness we also failed to achieve a response to selection in an upward direction in the present study (section 3.4.1.1.). Contrary to the DH line, the lack of response in the TH line was not accompanied by reduced fitness. The present study started with a base population that was probably already at the upper selection limit for territorial aggressiveness (section 4.4.2.). An array of selective forces

that act on territorial aggressiveness makes it plausible that territorial aggressiveness is kept within certain limits in nature. In view of our discussion in section 5.2.1. one would expect that the selective forces that act on brightness of colouration may also influence territorial aggressiveness. To these a number of selective forces may be added that act on the level of territorial aggression, either separately or in combination with the brightness of colouration.

In an ecological study on territory in the three-spined stickleback VAN DEN ASSEM (1967) found a positive correlation between aggressiveness (expressed as the number of attacks initiated by the owner) and territory size. As territory size was also positively correlated with courtship-success (expressed as the probability of getting a female in the nest) and parental-success (expressed as the number of eggs found at the end of the parental cycle), a relation between aggressiveness and courtship- as well as parental-success appears to be present. Comparable results with respect to aggressiveness and fitness were obtained in studies by LI & OWINGS (1978a, b).

The question arises whether the number of attacks initiated by the territory owner in a situation with several rival males is in anyway connected with territorial aggressiveness. The same holds for the studies by LI & OWINGS (1978a, b). Strictly speaking, the measures of aggressiveness in the above mentioned studies are dependent on the situation and not standardized measures of aggressive motivation. It remains therefore to be seen to what extent differences of aggressiveness in these studies correspond to individual differences of territorial aggressiveness, the more so as rank numbers (based on territory size) of males holding territories in a certain situation did not agree well with those found for the same males when re-tested in a different situation (VAN DEN ASSEM, 1967). In this context it may be mentioned that FITZGERALD (1983) found just the reverse relationship between aggressiveness and fitness for *G. aculeatus* in tidal salt marsh pools where three stickleback species breed sympatrically.

Besides the possible advantages of high levels of territorial aggression for three-spined stickleback males mentioned before, there are some disadvantages. First, there is a predation pressure not only against brightness of colouration, but also against the level of territorial aggression. HUNTINGFORD (1976a, b) found a positive correlation between the level of territorial aggression of three-spined stickleback males and the degree of boldness towards a small hunting pike (*Esox lucius*). An analysis of fish caught at sites with various predation pressures revealed that

sticklebacks coexisting with abundant predators showed the lowest level of both territorial aggression and boldness towards a pike (HUNTINGFORD, 1982: however, GILES & HUNTINGFORD, 1985, query the way in which the population differences in aggression were measured in this study). In populations with high predation risks males with high levels of territorial aggression may be more vulnerable to predation because of their boldness towards predators or else because their frequent involvement in fighting makes them more conspicuous as well as less vigilant. Secondly, it was shown in the present study that the genetic factors that contribute to variation of territorial aggressiveness and of courtship aggressiveness are at least partly identical (section 4.6.2.1.) and thus that high levels of territorial aggression coincide with high levels of courtship aggression. Too high a tendency to behave aggressively towards a gravid female will surely interfere with courtship (*e.g.* SEVENSTER, 1961; WILZ, 1972, 1973) and reduce the chance of successful mating.

The selective forces discussed in this section may hold territorial aggressiveness within certain limits in nature. Predation pressure is probably an important force in reducing territorial aggressiveness. In populations where predation pressure is low territorial aggressiveness is likely to reach an upper selection limit, which then is probably imposed by reduced courtship-success of males with too high aggression levels.

5.2.3. *Female and juvenile aggressiveness.*

The selective forces that act on female aggressiveness are less understood. The present study shows that the genetic factors that contribute to variation of female aggressiveness and of juvenile aggressiveness (of both sexes) are most likely identical (section 4.3.1. and 4.3.4.). In view of the conspicuousness of aggressive behaviour it is reasonable to assume that predation pressure is also exerted on juvenile and female aggressiveness. Some indirect indications for this are given by GILES & HUNTINGFORD (1984) in a study on inter-population variation in anti-predator responses (to a model heron and a live pike) in *G. aculeatus* males, females and fry from seven Scottish populations with various predation risks from fish and bird predators. Although they did not assess intra-specific aggression levels, some evidence for predation pressure on aggression may be deduced from that study, since in *G. aculeatus* intra- and inter-specific aggression appear to covary (HUNTINGFORD, 1976a, b, 1982). In both sexes and in fry there were significant differences between populations in anti-predator response, which could be related to differences in predation

risks at the study sites. In general, within each population females had better developed fright responses than adult males. It is interesting to note that the overhead fright response of wild-caught fry and laboratory-reared fry from the same stickleback population developed similarly (GILES, 1984). At an early age young showed a lower fright response than at a more advanced age, which would correspond to the decline of mean juvenile aggression with age in the present study (section 2.2., 3.3.3. and 4.6.1.). In a laboratory study of sexual selection on male and female three-spined sticklebacks LI & OWINGS (1978a) showed that females also compete intrasexually, and that they are also discriminating in mate selection. They observed that in groups of six females dominance relations were established, and that status was positively related to home range size. On two occasions higher status females caused spawning in subordinate females by quivering on them. When the group of females was given the opportunity to enter an adjacent group of six males (with at least two nests), then the dominant individuals had priority of access to the males and were more effective than subordinates at disrupting courtship by other females.

The present study indicates two other selection pressures that act on female aggressiveness. The first is the negative interference of high levels of female aggression with courtship-success, as observed to an extreme degree in some JH line females (section 3.3.1.). The second is the positive correlation between female aggressiveness and fecundity. Females of the JL line were less often ripe than JH females (section 4.3.1.). This difference was also found between TH and TL females (section 3.4.1.2.), though to a lesser extent.

Thus also with respect to female aggressiveness a number of selective forces can be recognized that may keep female aggressiveness within certain limits, and it is likely that other selective forces acting on aggressiveness in various contexts can be added to the above lists. By using lines selected for extreme aggressiveness in different social contexts, the functional aspects of aggressiveness can be more fully explored.

5.3. Hormonal influences.

In sticklebacks manipulation of the photoperiod, combined with castration and with administration of testosterone or gonadotropin has shown that before sexual maturity the level of aggression is associated with the level of gonadotropins (HOAR, 1962a, b; WAI & HOAR, 1963; BAGGERMAN, 1966, 1968). The level of aggression during the so-called sexual phase (*i.e.* the period after nest-building and before the start of the paren-

tal cycle, see Fig. 4 in SEVENSTER, 1961) is higher than before nestbuilding. The hormonal control of aggression in this phase is, however, less clear than before sexual maturity. When males are castrated nine to nineteen days after completion of the nest, then a significant decline of territorial aggression is noticed. The level reached is far lower than that of intact males just before sexual maturity, or that of males castrated one week before reaching this state. These results led to the hypothesis that in the sexual phase the hormonal control of aggression passes from gonadotropic hormones to the gonadal hormones (BAGGERMAN, 1966, 1968). This view has been criticized by WOOTTON (1970), who found that castration within one week after nestbuilding led to a level of aggression equal to that found just before nestbuilding. He suggests that the causal factors for aggression prior to nestbuilding (viz. gonadotropins) are still operating in the gonadectomized fish, and that there is not a total switch in the hormonal control of aggression after nestbuilding. The higher level of aggression found in unoperated fish is in his view either caused by additional gonadal hormones or to an aggression stimulating effect of the nest. There are indictions that a nest positively influences the level of aggression during the sexual phase. Removal of the nest leads to a decline in aggression (SYMONS, 1965). From SYMONS' data it is unfortunately not possible to assess the actual degree of the decline, but levels immediately after removal remain higher than before nestbuilding, suggesting an influence of gonadal hormones.

The present study revealed (a) that in the juvenile aggression lines, juvenile aggressiveness was negatively correlated with age of sexual maturity across the generation means (section 3.3.3.), and (b) that females of the high and low juvenile aggression lines differed in the incidence of ripeness: low line females became less often ripe than high line ones (section 4.3.1.). It is likely that the hormonal causation of juvenile aggressiveness in both sexes can be ascribed to gonadotropic hormones. Differences in the level of gonadotropins as a result of selection may be responsible for the above mentioned results. In view of the high genetic correlation of juvenile and female aggressiveness (section 4.3.1. and 4.3.4.) gonadotropic hormones can also be viewed as causal factors for female aggressiveness.

The present study also revealed (c) that selection for territorial aggressiveness most likely effected changes in the level of androgens in reproductive males (section 4.7), which were accompanied by changes in the brightness of colouration (territorial aggression decreased in the low line, but no increase was found in the high line), and (d) that most likely

no changes in the level of androgens nor in the brightness of colouration were effected in adult males of the juvenile aggression lines.

These results make it rather unlikely that gonadotropic hormones and the nest can be labelled as causal factors for territorial aggressiveness in the sexual phase (see WOOTTON, 1970). For if this were true, then one would expect (a) that juvenile males of the TL line would also show a clear reduction of juvenile aggression. However, the correlated response of juvenile aggression in TL males is weak (section 4.3.2.). Moreover, this correlated response may partly be effected by the simultaneous selection for female aggressiveness in the territorial aggression lines. Further one would expect (b) that reproductive males of the JL line would also show a clear reduction of territorial aggression. This expectation is not fulfilled either (section 4.3.2.) (females, on the contrary, do fulfill similar expectations, see section 4.3.1., as could be expected if their aggressiveness in both developmental stages is controlled by gonadotropic hormones). We cannot exclude some influence of gonadotropins on territorial aggression, since JL males show some indication of a reduced territorial aggression level (section 4.3.2.), though judging from their kidney sizes, most likely no changes in the level of androgens have occurred (section 4.7.). This seems to argue against BAGGERMAN's hypothesis that gonadotropic hormones do not play a role at all in the control of territorial aggression. Another point that argues against the idea that aggression in the sexual phase is solely controlled by androgens, is the lack of response to selection for high territorial aggressiveness, though most likely the level of androgens does change in the TH line. This in itself is in favour of WOOTTON's idea that androgens do not play a role in determining the level of aggression in the sexual phase. But as we have seen such a view is not in accordance with other facts. The best explanation seems to be that both androgens and gonadotropic hormones play a role in the causation of territorial aggression during the sexual phase, with an additional (immediately acting) influence of nest stimuli. The role of gonadotropic hormones seems less important than that of gonadal hormones. Furthermore, we have to assume some kind of negative feedback of high levels of androgens on the synthesis and/or release of gonadotropic hormones in order to explain the fact that no increase of aggressiveness is found in TH males. The brightness of colouration seems to depend here on androgens. If one selects for enhanced territorial aggressiveness the level of androgens most likely increases, but in such a relation to gonadotropins that the net result expressed in aggressive behaviour does not change. Natural selection has apparently acted on a

certain maximum of territorial aggression based on a stabilized outcome of interacting hormones.

Since the genetic factors that contribute to variation of territorial and of courtship aggressiveness are probably identical (section 4.6.2.1.), the same hormones may control courtship aggressiveness. Lastly, variation of dominance ability is probably governed by genetic factors different from those governing variation of aggressiveness in the other situations (section 4.3.3.). The probably unchanged levels of androgens in the dominance lines (section 4.7.) suggest that here androgen-independent factors (or possibly factors that effect a change in sensitivity to androgens) that influence the brightness of colouration determine the level of dominance ability.

The identity of the gonadotropic hormones in teleosts is a matter of controversy. It is doubtful whether two different gonadotropic hormones can be discerned, analogous to the situation in higher vertebrates, or only one with both FSH and LH properties (see *e.g.* BENTLEY, 1982). This uncertainty also applies to the three-spined stickleback. AHSAN & HOAR (1963) could stimulate spermatogenesis and development of secondary sexual characters in males kept on short day-lengths with mammalian LH, but not with FSH. In females both functions could also be stimulated with LH alone. The level of pre-breeding aggression of males kept on short day-lengths could be enhanced by mammalian LH, but not by FSH (HOAR, 1962a, b). These results suggest that only one gonadotropic hormone exists in sticklebacks. However, in HOAR's experiments reproductive behaviour was never fully stimulated and treated females only rarely spawned (HOAR, 1962b). Furthermore, SLIJKHUIS (1978) presented ultrastructural evidence (which is, however, not generally accepted as such; BORG, pers. comm.) for two types of gonadotropic cells in the pituitary gland of the male three-spined stickleback. One cell type was active both in immature males in which the testis development is completed as well as in mature males. The other cell type was only active in mature males.

The data generated by the present study may suggest the existence of two gonadotropins, since (a) the obvious difference in juvenile aggressiveness between JH and JL males (which is assumed to reflect a difference in level of gonadotropic hormones) does not effect a difference in level of androgens when the males become sexually mature (section 4.7.). Further, since (b) the obvious difference in level of androgens between TH and TL males is not reflected in an appreciable difference of juvenile aggressiveness between TH and TL juvenile males (section 4.3.2.). With

respect to juvenile aggression in the juvenile aggression lines it must be noted that the aggression scores are based on tests before day 98 (after fertilization). Juvenile tests more close to sexual maturity, however, still reveal a large difference in aggressiveness between high and low line fish (section 3.3.3. and 4.6.1.).

Though suggestive for the existence of two gonadotropins, namely one controlling the level of juvenile aggression (and female aggression), the other the production of androgens (not selected for in the JH and JL line), the two facts given above do not necessarily support such an interpretation. They may also be explained by a "one-gonadotropic hormone" hypothesis.On the basis of this latter hypothesis the following may be suggested with respect to the events occurring in males of the juvenile aggression lines. With a reduced level of gonadotropic hormone JL males may eventually produce an androgen level that is indiscernible from the control level, though it will take more time to reach such a level. On the other hand, in the JH line the production of androgens, which in JH males is not different from the control either, may reduce by negative feedback the level of the gonadotropic hormone to control values during the sexual phase (if there is a question of enhanced gonadotropin level at all, as the response in the JH line is doubtful, see section 3.3.1.). In this line of thought, in JL males a delay of the onset of sexual maturity is to be expected, as actually has been found (section 3.3.3.). If one accepts that territorial aggressiveness is mainly controlled by androgens, then slight differences in the level of gonadotropin will have no effect on territorial aggressiveness. Judging from the result of selection (and accompanying changes in the age at sexual maturity and in incidence of ripeness), one expects a clear reduction of the level of gonadotropic hormone in JL fish, and thus some deviation from the control level of territorial aggression. Though not significant, such a decrease is seen in territorial aggression of reproductive JL males (section 4.3.2.) as well as in their courtship aggression (section 4.6.2.1.). On the basis of the one-gonadotropin hypothesis the following may be suggested with respect to the events occurring in males of the territorial aggression lines. Since juvenile aggressiveness is not different between high and low line juveniles, the decrease of territorial aggressiveness in TL males (section 3.4.1.1.) may be interpreted as being due to a decrease in androgen level (possibly because of a decrease in sensitivity of testis tissue to gonadotropic hormone). In TH males an increase in sensitivity to gonadotropin is assumed to be the effect of selection for high territorial aggressiveness. Nevertheless an increase of territorial aggression fails to

appear in TH males, because of an increased effect of negative feedback on the level of gonadotropin. The additional effect of gonadotropin on territorial aggression is probably considerably reduced in these males. On the other hand, the enhanced level of androgens expresses itself in a brighter colouration relative to C males.

In summary, discrepancies between gonadotropic and gonadal hormone levels in high and low line males of the juvenile and territorial aggression lines might be interpreted on the basis of a one- or two-gonadotropic hormone hypothesis. More endocrinological research has to be carried out in order to disentangle the complex relationships between the levels of hormones responsible for aggressiveness.

Under the applied experimental conditions juvenile aggressiveness follows a characteristic course as a function of age (section 3.3.3. and 4.6.1.), a topic that would be interesting for future behaviour-endocrinological research. A compound that might influence the juvenile aggression profile of three-spined sticklebacks is melatonin. VAN VEEN *et al.* (1980) have shown that the pineal complex of the three-spined stickleback is most likely capable of photoneuroendocrine transduction. Melatonin could induce antigonadal effects in both sexes of the three-spined stickleback kept on long day-lengths (BORG & EKSTRÖM, 1981). This might be effected by the influence of melatonin on the synthesis/release of antigonadotropic peptides in the pineal organ. The disappearance of the characteristic dark cross-bars in the skin of young of the juvenile aggression lines suggests a possible influence of melatonin in the present study. In a number of isolated JH-3 and JL-3 juveniles (of both sexes) these bars temporarily disappeared completely or almost completely, and the young then appeared uniformly silver. There are some indictions that the first peak in the incidence of silvery young coincides with the reduced level of juvenile aggression after the first aggression peak in groups of young, that is in the 10-th and 11-th week after fertilization (see section 4.6.1.). This variation in the visibility of cross-bars might reflect a variation in melatonin activity.

Summary

This behaviour-genetic study concentrates on intra-specific aggressiveness in the three-spined stickleback (*Gasterosteus aculeatus* L., forma *leiura*). Aggressiveness was studied under standardized conditions in five different test situations, referred to as juvenile aggressiveness, female aggressiveness, territorial aggressiveness, courtship aggressiveness, or dominance ability. The aim of the study is two-fold:

1. To assess the extent to which variation of aggressiveness in each of the different test situations is attributable to genetic causes.

2. To assess the extent to which variation in these various manifestations of aggressiveness is influenced by common genetic factors.

The paper starts with an analysis of the variation of aggressiveness in the base population, composed of individually isolated progeny of wild-caught parents (chapter 2). In each of the different test situations aggressiveness is highly variable across individuals. Repeated measurements with the same individuals as well as similarity of the levels of aggression between full sibs shows that phenotypic variation is to a considerable extent attributable to genotypic variation in each of the investigated situations.

The genetic influence on variation of aggressiveness is further analysed with the aid of selection experiments (chapter 3). Bidirectional selection is exerted upon juvenile aggressiveness in juveniles of both sexes (juvenile aggression lines), upon territorial aggressiveness in males and female aggressiveness in adult females (territorial aggression lines), and lastly, upon dominance ability in males (dominance lines). Besides these six selection lines an unselected control line was maintained.

Selection is highly successful in downward direction in each of the different contexts. However, enhancement of the level of aggression is less successful in most lines, with the exception of female aggressiveness. Possible causes for these asymmetries of responses are discussed in detail. It is argued that the lack of response in males to selection for territorial aggressiveness and dominance ability in upward directions is probably due to an upper selection limit for territorial aggressiveness and dominance ability, that is already present in the base population. The combined two-way responses yield heritability-estimates of at least 0.30 for aggressiveness in the different test situations. In the single selection lines the realized heritabilities range from 0 to at least 0.60.

Across individuals of the base population the levels of aggression in the different test situations are correlated with each other to various degrees. In females there is a distinctly positive phenotypic correlation between juvenile and female aggressiveness, but in males correlations between juvenile, territorial and courtship aggressiveness are weaker. Dominance ability is uncorrelated with aggressiveness in the other test situations.

Since fish of the various selection lines are not only scored for their aggressiveness in the particular situation in which they are selected, but also for their aggressiveness in the other test situations, these phenotypic correlations can be translated into genetic correlations (chapter 4). The genetic correlations between the levels of aggression in the different test situations are comparable to the corresponding phenotypic correlations. The genetic basis of juvenile aggressiveness is most likely identical for both juvenile males and juvenile females. In adult females variation of aggressiveness remains most likely governed by the same genetic factors. The genetic factors that contribute to variation of territorial aggressiveness are only partly identical to those that contribute to variation of juvenile aggressiveness. Changes of courtship aggressiveness parallel those of territorial aggressiveness in the various selection lines, suggesting that common genetic influences underlie variation in both manifestations of intra-specific aggressiveness in reproductive males. Lastly, variation of dominance ability is probably governed by genetic factors different from those governing variation of juvenile or territorial aggressiveness.

Indirect determination of the level of androgens in reproductive males of the various selection lines, by means of kidney-size measurements, reveals that selection for territorial aggressiveness most likely acts on the level of androgens. In view of changes accompanying selection for juvenile aggressiveness (viz. a difference between high and low line fish in age at sexual maturity, in age at onset of juvenile aggression, and in incidence of ripeness) selection for juvenile aggressiveness likely acts on the level of gonadotropic hormones. Finally, selection for dominance ability acts on factors (probably androgen-independent) that influence the brightness of colouration.

Throughout this paper the influence of a number of variables on aggressiveness in the various test situations, such as age, degree of ripeness, location of the nest, age of sexual maturity, experience, length, isolation and responsiveness, are analysed. Furthermore,

attention is paid to threatening displays, to a peculiar behaviour called snapping at air-bubbles, to aggressiveness of juveniles in groups of fixed size, and to measures of aggressiveness other than the criterion applied during selection in a particular situation. Finally, the literature is reviewed with respect to selection studies on aggressiveness and to selective forces acting on aggressiveness in the three-spined stickleback.

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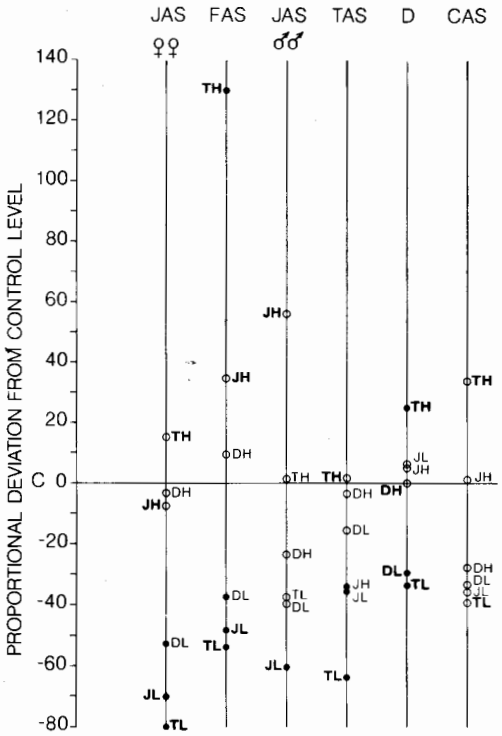
Appendix



Responses (in lines indicated to the left of the vertical bars) and a number of correlated responses (in lines indicated to the right of the vertical bars) after three generations of selection for aggressiveness. Responses and correlated responses are expressed as proportional deviations from the control level (mean of C-0 and C-2). Lines printed in bold type indicate a significant ($p < 0.05$) difference between H and L (Mann-Whitney U test, 2-tailed, or in the case of D, χ^2 test, 2-tailed). ● = indicates a significant ($p < 0.05$) deviation from C-2 (Mann-Whitney U test, 2-tailed) or, in the case of D, from DH-3 (tested with χ^2 test, 2-tailed). JAS = juvenile aggression score, FAS = female aggression score, TAS = territorial aggression score, CAS = courtship aggression score, D = dominance ability, JH = high juvenile aggression line, JL = low juvenile aggression line, TH = high territorial aggression line, TL = low territorial aggression line, DH = high dominance line, DL = low dominance line, C = control line.