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Changes in the sexual tendency accompanying selection for aggressiveness in the three-spined stickleback, *Gasterosteus aculeatus* L.*

Th. C. M. BAKKER† AND P. SEVENSTER

Ethology Research Group, Zoological Laboratory, University of Leiden, P.O. Box 9516, 2300 RA Leiden, The Netherlands

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Independent selection experiments for reduced juvenile and reduced territorial aggression levels in male three-spined sticklebacks both resulted in reduced courtship aggression levels. In the low juvenile aggression line this is accompanied by an enhanced sexual activity, but in the low territorial aggression line males display a reduced sexual activity. These results are consistent with a mutually inhibitory relationship between the aggressive and sexual tendencies, if selection for juvenile aggressiveness has only affected the aggressive tendency while selection for territorial aggressiveness has simultaneously affected the aggressive and sexual tendencies in the same direction. This is in agreement with the hormonal changes (pituitary-gonadal axis) suggested by the selection studies.

I. INTRODUCTION

In the male three-spined stickleback, *Gasterosteus aculeatus* L., behavioural studies have shown that there exists a mutually inhibitory relationship between the tendency to behave aggressively towards a stimulus and the tendency to behave sexually over a short time period and within an individual male (Sevenster, 1968, 1973; Peeke, 1969; van den Assem & van der Molen, 1969; Wilz, 1972). There is also evidence for this relationship in other teleost fishes such as the pumpkinseed, *Lepomis gibbosus*, (Ballantyne & Colgan, 1978), the bluegill sunfish, *Lepomis macrochirus*, (Ballantyne & Colgan, 1978), the Siamese fighting fish, *Betta splendens*, (Figler *et al.*, 1973, 1975; Klein *et al.*, 1976), the convict cichlid *Cichlasoma nigrofasciatum* (Cole *et al.*, 1980), and the cichlid *Aequidens portalegrensis* (Baerends, 1984).

Within an individual male this inhibitory relationship between the aggressive and sexual tendencies is evident at two different levels. First, there exists a moment-to-moment (or phasic) effect when aggressive and sexual behaviours are patterned on a fine time-scale: behaving aggressively inhibits behaving sexually, and vice versa (e.g., Sevenster, 1973). Second, there exists a tonic effect when individual levels of aggression and sex, averaged over a longer time period, are compared across tests (e.g., Peeke, 1969). Within an individual male, both the phasic and tonic effects presumably reflect some immediate causal organization of behaviour.

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†Present address: Universität Bern, Ethologische Station Hasli, Abteilung Verhaltensökologie, Wohlenstrasse 50a, CH-3032 Hinterkappelen, Switzerland.

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There is also a negative correlation between aggressive and sexual tendencies when these tendencies, averaged over a particular period of time (tonic effects), are compared across individuals in the same test situations (e.g. Sevenster, 1961): individuals which have a greater tendency to behave aggressively towards a stimulus have a smaller tendency to behave sexually towards that or other stimuli, and vice versa. In addition to the organizational causes for the negative relationship between aggressive and sexual tendencies within an individual, at the inter-individual level we may have to deal with environmental and genetic causes for the observed negative phenotypic correlation between these tendencies. Here we show that such phenotypic correlations are not necessarily paralleled by similar genetic correlations, i.e. there can be both negative and positive relationships between the genetic factors that relate to aggressive behaviour and the factors that relate to sexual behaviour.

Two pairs of artificial selection lines, selected over several generations for aggressiveness, were used to investigate this: the high and low territorial aggression lines (TH and TL lines) selected for territorial aggressiveness, and the high and low juvenile aggression lines (JH and JL lines) selected for aggressiveness during the juvenile stage. As a correlated response to selection, the low lines displayed a reduced level of aggression during courtship against ripe females, as compared with that of their corresponding high lines (Bakker, 1986). The sexual tendency of the selection lines' males will be deduced from their courtship and/or nest activities in two different situations: during courtship tests (test fish: an enclosed ripe female) and during observations of spontaneous activity (without any test fish).

The interesting point of this comparison is the possibility that different hormonal changes occur in the juvenile and territorial aggression lines. It has been argued, based on circumstantial evidence, that selection for juvenile aggressiveness has acted on the level of gonadotropic hormones, and selection for territorial aggressiveness on the level of androgens (Bakker, 1985, 1986). Differences in courtship aggressiveness between JH and JL males probably have a different hormonal causation than differences between TH and TL males (Bakker, 1986).

II. MATERIALS AND METHODS

SELECTION LINES

The sticklebacks used for this study were third and fourth generation males of two pairs of selection lines, the high and low juvenile aggression lines (JH and JL lines) and the high and low territorial aggression lines (TH and TL lines). The JH and JL lines were selected for high or low juvenile aggressiveness in both juvenile males and females, and the TH and TL lines for high or low territorial aggressiveness in males and high or low female aggressiveness in females. Besides the selection lines, an unselected control line (C line) was maintained. For further details, see Bakker (1986). The lines had been founded with fish from a freshwater population near Vaassen (The Netherlands) which is monomorphic for the forma *leiura*.

REARING CONDITIONS

The fish were kept under highly standardized conditions: the young were isolated (physically and visually) at an early age (generation 3, 3 weeks after fertilization; generation 4, 6–9 weeks after fertilization) in plastic tanks (34 × 17 × 20 cm) which were set up in a standardized way and placed in an air-conditioned room (temperature 18–20° C) with long daylength (16L:8D). They were fed twice a day on live and/or frozen food (Bakker, 1986).

GENERATION 3 TESTS

After the males had completed their first nest, they were submitted once a week in four successive weeks to male tests (especially designed to test territorial aggression, hence called T tests) and courtship tests (C tests) in their rearing tanks. T tests and C tests were separated by at least one day.

In the T tests, a rival male was offered in a polyacrylic chamber ($6 \times 6 \times 20$ cm) with opaque side walls. The chamber was hung outside at the front of the tank as far as possible away from the nest. A complete recording of the behaviour of the experimental male was made for 5 min from the moment he reached the chamber. Then the chamber was removed. Aggressiveness in the T test was expressed as the total duration of biting and bumping at the opponent as a percentage of the 5-min test period. Individual aggression scores were obtained by averaging for each fish the results from the series of T tests. These average scores were called territorial aggression scores (TAS).

In the C tests, a ripe female, selected for her readiness to adopt and maintain the courtship posture, served as a test fish. She was offered in a glass tube (4·5 cm diameter) as far as possible away from the male's nest, inside his tank, but not in contact with the walls. Individual aggression scores obtained during C tests were called courtship aggression scores (CAS).

The numbers of males tested are indicated in the figures. It must be noted that the number of TL-3 males tested is limited because of a female-biased sex ratio in the TL line (details are given in Bakker, 1986).

GENERATION 4 TESTS

Testing of generation 4 males occurred in larger tanks ($60 \times 40 \times 40$ cm) to which the males were transferred usually 1–2 weeks after completion of the first nest in their rearing tanks ($34 \times 17 \times 20$ cm). The tanks were set up in a standard manner with a single row of long-leafed plants (*Vallisneria, Allisma,* etc.) against the back wall, and some tufts of green filamentous algae. They were filled with equal parts of tap water and de-ionized water. Each tank was illuminated by a 100-W bulb about 20 cm above the water surface. The light regime, temperature and diet were the same as in the smaller rearing tanks.

During the first week in the experimental tanks the males were stimulated daily with a ripe female enclosed in a glass tube, and when the nest was completed they were submitted to four series of behavioural tests in the next two weeks. Each of the series consisted of a T test (with a short after-effect: Sevenster, 1961) followed, after 10 min, by a 1-h observation of the male's spontaneous activity, and a C test. The observer was uninformed about the origin of the males to be tested.

The T and C tests deviated from those in generation 3 in the following four ways:

(1) rival males were offered in glass tubes of 6 cm diameter and ripe females in tubes of 7 cm;

(2) rival males were, on average, offered closer to the nest, at a distance of 10 cm from the nest entrance instead of 10–30 cm, and ripe females were, on average, offered further away from the nest, at a distance of 27 cm from the nest entrance instead of 15–25 cm, the test fish (male or female) being always offered at the same place in tests of a particular male;

(3) the duration of tests was 3 min instead of 5 min;

(4) the aggression scores were not based on the duration of biting and bumping, but on the number of bites and bumps in the tests, and are denoted as 'TAS' and 'CAS', the inverted commas indicating this difference.

The number of generation 4 males tested are indicated in the figures. Unfortunately, for technical reasons no C line and only a very limited number of JL line males were available at the time of testing.

MEASURES OF THE SEXUAL TENDENCY

Several measures were scored to determine the sexual tendency. At a fine level of analysis these measures can be shown to compete with one another (e.g., Cohen & McFarland, 1979) but all of these measures can be used as indicators of sexual tendency on a longer time scale.

Descriptions and definitions of the behavioural elements of the male three-spined stickleback in the sexual phase are given in van Iersel (1953), Sevenster (1961) and Wootton (1976). Courtship is best regarded as an ambivalent behaviour in which aggressive and

sexual elements are interacting. Leading to the nest is a purely sexual activity and the frequency of leading in C tests would be an appropriate measure of the sexual tendency. However, the frequency of leading is often low or even zero, especially when the female is offered relatively close to the nest. The frequency of leading is closely correlated with the frequency of zigzagging (Sevenster, 1961; Sevenster-Bol, 1962; Rowland, 1982). Since the number of zigzags can accurately be counted and has a greater range than the frequency of leading, and since zigzagging also occurs in tests in which no leading is displayed, the frequency of zigzagging is usually taken as a measure of the sexual tendency (in spite of the ambivalent nature of zigzags) and was used as such.

Another indicator of the sexual tendency used in this study was the probability of the male creeping through his nest (CT), since this probability is closely linked with the after-effect of courtship. After courtship, when the female has disappeared, the probability of CT diminishes. The waning of the sexual tendency is parallelled by the decline in the probability of CT (Sevenster, 1961; Nelson, 1965; 't Hart, 1978). Some males regularly crept through two or three times in quick succession (an aberration that is expressed when fish are reared under long daylength; Sevenster & 't Hart, 1974). These multiple passages through the nest were counted as a single CT.

As a third indicator of the sexual tendency, the frequency of nest visits was used, since the frequency of CT and the number of zigzags in C tests are positively correlated with the number of nest visits (Sevenster & van der Sanden, in prep.).

III. RESULTS

Males from both the low territorial and low juvenile aggression lines had reduced levels of territorial aggression: TL males as a direct response to selection for low territorial aggressiveness, and JL males as a correlated response to selection for low juvenile aggressiveness. In the high lines, selection was ineffective (Bakker, 1985, 1986).

Changes in courtship aggressiveness parallel those of territorial aggressiveness in the selection lines (Bakker, 1986). This was shown by the positive correlation between courtship aggressiveness and territorial aggressiveness across the means of the first three selected generations of the TH, TL, JH, and JL lines, and of the C line (Fig. 1). The low mean CAS and TAS values of TL-3 and JL-3 are indicated in the plot. Across the generation means of the fourth selected generations a similar correlation ($r_s = 0{\cdot}60$) existed between territorial and courtship aggression scores. Throughout the selection experiments, the JL and TL lines consistently gained lower mean courtship aggression scores than their corresponding high lines or the C line. The difference between the low and high line was significant for the combined first three selected generations in both pairs of selection lines (Mann–Whitney U test, 1-tailed; JH *v.* JL, $P < 0{\cdot}01$; TH *v.* TL, $P < 0{\cdot}05$). However, due to relatively low levels of aggression during courtship (Fig. 1) and low numbers of males tested, the level of significance was not reached for most of the separate generations (Mann–Whitney U test, 1-tailed: JH-3 *v.* JL-3, $P > 0{\cdot}10$; TH-3 *v.* TL-3, $P < 0{\cdot}05$; JH-4 *v.* JL-4, $P > 0{\cdot}10$; TH-4 *v.* TL-4, $P = 0{\cdot}10$).

Intra-line comparisons of the levels of aggression and the sexual activities during C tests on generation 3 males suggested a mutually inhibitory relationship between the aggressive and sexual tendencies in each of the lines, because the relative duration of biting and bumping during C tests was negatively correlated with measures of the sexual tendency (Table I).

On account of the comparably low levels of courtship aggression of the JL and TL lines, we expected similar sexual tendencies in both low lines. An analysis of the

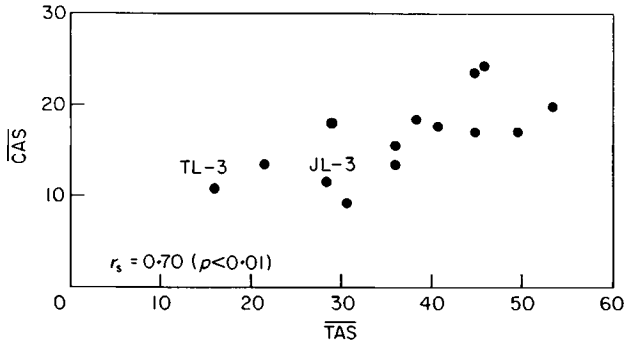

FIG. 1. The correlation of the mean courtship aggression score (CAS) with the mean territorial aggression score (TAS) across the first three selected generations of the juvenile and territorial aggression lines, and the control line, of three-spined sticklebacks. JL-3, generation 3 of the low juvenile aggression line; TL-3, generation 3 of the low territorial aggression line; r_s, Spearman rank correlation coefficient.

TABLE I. The correlation of three-spined stickleback courtship aggression (expressed as the % biting-bumping time in the test-period) with some measures of the sexual tendency during courtship tests on third generation males of the juvenile and territorial aggression lines

Line	Number of tests	r_s* between courtship aggression and		
		Number of zigzags	Frequency of nest visits	% at nest
JH-3	63	−0·61	−0·71	−0·77
JL-3	32	−0·69	−0·75	−0·69
TH-3	63	−0·62	−0·57	−0·65
TL-3	28	−0·40	−0·43	−0·43

*r_s, Spearman rank correlation coefficient. All correlation coefficients differ from 0 at the 5% level.

sexual tendencies of generation 3 males showed that this expectation was not met: JL-3 and TL-3 males did differ with respect to their sexual tendency. This was indicated by the incidence of creeping through (CT) during C tests and during stimulation by a short, *c.* 1 min, presentation of a ripe female in a glass tube (the males were stimulated once or twice a week in the period between completion of the tests and breeding to keep them in good reproductive condition) (Fig. 2, left part). In a very general way, the incidence of CT is closely linked to the sexual tendency and can be used as an indicator of this tendency (Sevenster, 1961). The different sexual tendencies of JL-3 and TL-3 males were due to a trend towards enhanced sexual tendencies in JL-3 males and a trend towards reduced sexual tendencies in TL-3 males. So the reduced courtship aggressiveness in the JL line tended to be accompanied by an enhanced sexual tendency, while a similar reduction in courtship aggressiveness of TL males tended to be accompanied by a reduced sexual tendency as well. Similar, but less apparent, trends were found for the frequency of zigzagging.

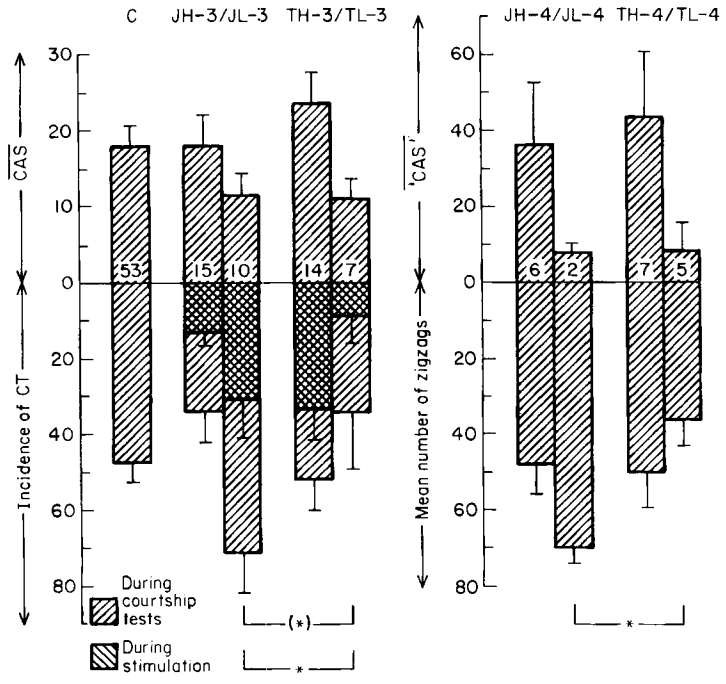

FIG. 2. Left: three-spined stickleback mean aggression scores ($\overline{CAS}$) and incidence of creeping through (CT) during courtship tests on control line males (generation 0 + generation 2) and third generation males of the juvenile and territorial aggression lines, and incidence of CT during stimulation of generation 3 males of the selection lines; the incidence of CT is expressed as the mean % of total tests per male in which CT occurred. Right: mean aggression scores ('$\overline{CAS}$') and mean number of zigzags during courtship tests on generation 4 males of the juvenile and territorial aggression lines. Lengths of bars represent one standard error of the means. Number of males tested is indicated. (*), $P<0{\cdot}10$; *, $P<0{\cdot}05$ (Mann Whitney U test, two-tailed for generation 3, 1-tailed for generation 4).

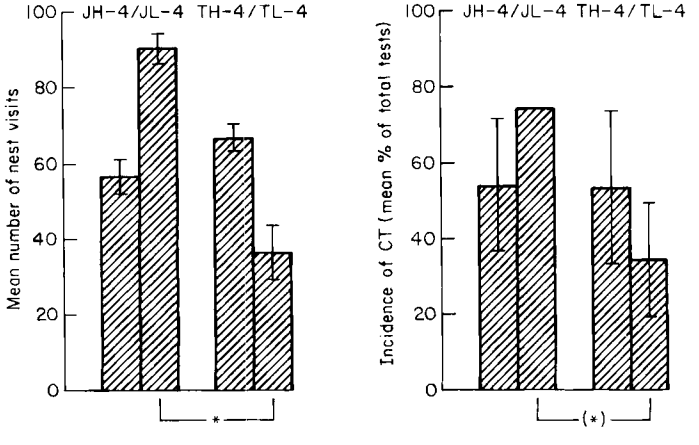

FIG. 3. Mean number of nest visits and incidence of creeping through (expressed as the mean % of total tests per male in which CT occurred) during observations of spontaneous activity on fourth generation males of the juvenile and territorial aggression lines of three-spined sticklebacks. Lengths of bars represent two standard errors of the means. (*), $P<0{\cdot}10$; *, $P<0{\cdot}05$ (Mann–Whitney U test, one-tailed).

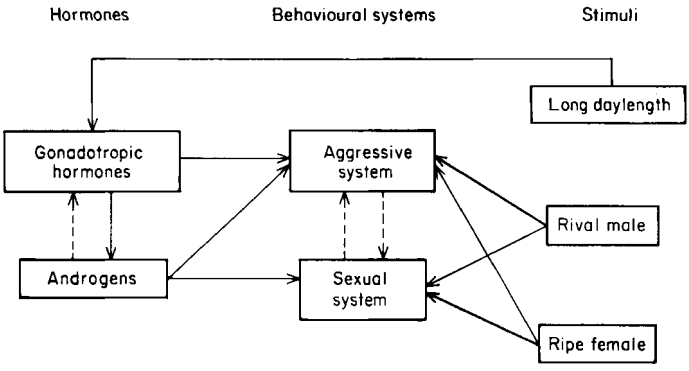

FIG. 4. Representation of the interrelations between the aggressive and sexual systems of the male three-spined stickleback in the sexual phase. Continuous lines represent positive effects, dotted lines negative effects. The thick continuous lines indicate the main effects of the external stimuli.

The small tanks in which generation 3 males were tested probably inhibited the full expression of courtship behaviour. To verify the observed trends in the sexual tendency, generation 4 males were tested in larger tanks, in which the female could be offered at a greater distance from the nest. The frequency of zigzagging during C tests on generation 4 males indicated a significant difference between the sexual tendencies of JL-4 and TL-4 males: the frequency of JL-4 males being twice that of TL-4 males (Fig. 2, right part). Observations of generation 4 males during the sexual phase without any test fish confirmed this. Differences in the sexual tendency were expressed in differences in the incidence of CT and in the frequency of nest visits. Again we know that, in a general way, these activities are closely linked to the sexual tendency (Sevenster, 1961). JL-4 males scored significantly higher than TL-4 males for these measures of the sexual tendency (Fig. 3). These data, combined with those from C tests on generation 3 and generation 4 males, show that JL and TL males differed in their sexual tendency in spite of identical low levels of courtship aggression, due to a trend towards a reduced sexual tendency in TL males as opposed to a trend towards an enhanced sexual tendency in JL males.

IV. DISCUSSION

Artificial selection for low juvenile aggressiveness and for low territorial aggressiveness resulted in reduced aggression levels during courtship, as compared with their corresponding high lines or with the control line. Within each line a mutually inhibitory relationship between the aggressive and sexual tendencies in the short term was found inter-individually. An analysis of the sexual tendencies revealed that both low lines differed significantly in that males of the JL line tended to show enhanced sexual activities, while a tendency towards reduced sexual activities was found in TL males. The changes in the juvenile aggression lines as a result of selection may be explained without assuming a genetic correlation between aggressive and sexual tendencies; the changes being a result of selection for genetic factors that merely affect the aggressive tendency. As a correlated response to selection for juvenile aggressiveness in a downward direction, JL males have a reduced courtship aggressiveness, and, as a secondary effect (the negative

relationship between aggression and sex within an individual) this enhances their sexual tendency. However, the existence of an overall negative genetic correlation cannot be precluded on the basis of these results. In the territorial aggression lines, the low line males tended to show a reduced sexual activity. Selection for territorial aggressiveness in a downward direction not only effects a reduced aggressiveness during courtship but simultaneously reduces the sexual tendency. So both the aggressive and sexual tendencies are affected in the same direction. The changes in the territorial aggression lines are a result of selection for genetic factors which cause a positive correlation between aggressive and sexual tendencies.

The different sexual tendencies of TL and JL males are indicative of a difference in the causation of the reduction in courtship aggressiveness in the two low lines. The JL line was selected for low juvenile aggressiveness. It has been argued, based on circumstantial evidence, that this selection has acted on the level of gonadotropic hormones. The TL line was selected for low territorial aggressiveness and this selection has most likely acted on the level of androgens (as assessed indirectly; Bakker, 1985, 1986). The reduced courtship aggressiveness is probably caused by a reduced level of gonadotropins in JL males and by a reduced level of androgens in TL males. From the changes in the aggressive and sexual tendencies and the probable hormonal changes in JL and TL males, it is deduced that gonadotropic hormones influence the aggressive tendency but not the sexual tendency, and that androgens influence both the aggressive and sexual tendency in the same direction.

A hypothetical scheme for the influences of the pituitary-gonadal axis on the motivational systems for aggression and sex of the male three-spined stickleback in the sexual phase is presented in Fig. 4. The secretion of gonadotropic hormones is stimulated by long daylength (e.g., Baggerman, 1957) which, in turn, stimulates the production of androgens. In the sexual phase, androgens (and probably some non-steroidal factor from the testes) exert a negative feed-back on the gonadotropic cells (Borg *et al.*, 1985). The pituitary-gonadal axis is here considered to modulate the aggressive and sexual systems in such a way that both gonadotropic and gonadal hormones have a direct influence on the aggressive system, while the sexual system is only influenced directly by the gonadal hormones. Furthermore, there is a mutually inhibitory relationship between the aggressive and sexual systems, causing a negative intra-individual correlation between aggression and sex in the short term. Indications for the involvement of gonadotropins in the control of aggressiveness during the sexual phase were provided by Hoar (1962*a, b*), Wootton (1970) and Bakker (1986), although Baggerman (1966, 1968) was doubtful about this role of gonadotropins during the sexual phase (discussed in Wootton, 1976, and Bakker, 1986). The effects of castration or anti-androgen treatment, and the reversibility of these effects by methyl-testosterone, have indicated the involvement of androgens in the control of aggressiveness during the sexual phase (Hoar, 1962*a, b*; Wai & Hoar, 1963; Baggerman, 1966, 1968; Wootton, 1970; Rouse *et al.*, 1977). Their experiments have also indicated the involvement of androgens in the control of the sexual tendency. This simultaneous action of androgens causes a positive correlation of the aggressive and sexual tendencies, if one compares these tendencies across periods of the life cycle with different androgen levels (both tendencies are for instance at their peak in the sexual phase) or across individuals (populations) with different androgen levels (as Rowland, 1984, probably did). Rival males and ripe females often elicit both

aggressive and sexual behaviour, though in different proportions. Aggressive behaviour is the main activity upon exposure to a rival male, and sexual behaviour upon exposure to a ripe female. Besides eliciting the behaviour, the stimuli influence also the aggressive and sexual tendencies themselves. With respect to the aggressive stimulus, the after-effect on the aggressive tendency is very short (less than 5 min; Sevenster, 1961), but after removal of a stimulus that elicits sexual behaviour it may take several hours before the sexual tendency has returned to its spontaneous level (Sevenster, 1961; Nelson, 1965; 't Hart, 1978).

The model presented in Fig. 4 deviates from that of Munro & Pitcher (1983) which summarizes the possible mechanisms by which the pituitary-gonadal axis influences aggressive behaviour in the male three-spined stickleback. Based on Wootton's (1970) suggestion, they consider nest building to mediate the effects of androgens on aggressiveness, since castration brings to an end and prevents nest building and nest activities. In this view, androgens do not exert a direct influence on aggressiveness. Although there is an (immediately acting) influence of nest stimuli (e.g., van Iersel, 1958) it is very unlikely that the presence of a nest is the only causal factor for the enhanced aggression levels during the sexual phase (Bakker, 1986).

Despite differences between the test conditions of generation 3 and 4 males, the interline differences of the aggressive and sexual tendencies are consistent (cf. Wootton, 1971). This emphasizes once again the genetic contribution to these differences. The interline differences in the sexual tendency tend to be more pronounced in generation 4 than in generation 3 males. On the one hand we are inclined to ascribe this to the progress of selection. On the other hand, the greater dimensions of the tank and the presentation of the female at a greater average distance from the nest in generation 4 has undoubtedly facilitated the full expression of courtship behaviour.

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