ADVANCES IN THE STUDY OF BEHAVIOR, VOL. 23

Genetic Correlations and the Control of Behavior, Exemplified by Aggressiveness in Sticklebacks

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I. GENETIC CORRELATIONS AS A TOOL IN STUDYING THE CONTROL OF BEHAVIOR

Many behavioral traits show continuous variation in a population. Such quantitative traits are under polygenic control, that is, are influenced by many genes of which each has a small effect on the phenotype. The continuous variation of quantitative traits is the result of their multifactorial inheritance on which is superimposed additional variation caused by environmental influences. These two causes of variation make the translation of genotype into phenotype rather indirect and leave much room for secondary influences on the expression of quantitative traits. The many genes involved in the long and indirect path from genotype to phenotype do not operate in isolation and each of them is amenable in varying degree to environmental influences. For a particular behavioral phenotype each step (gene) in this path may be influenced by genes that control other phenotypes. The interaction between genes that control different phenotypes can be very direct; the most extreme case being a gene that affects more than one phenotype, and is thus directly involved in the expression of different phenotypes. Geneticists call this pleiotropic gene action. Genes that are involved in the control of different phenotypes can also influence one another in more indirect ways, for instance, through nonrandom association of alleles at different loci, which is denoted by the technical term linkage disequilibrium. This disequilibrium between genes that are involved in the control of different phenotypes may be the direct consequence of physical linkage, thus decreasing the chance that recombination breaks down the gene associations, or may be upheld by selective forces that favor particular combinations of genes. Pleiotropy

and linkage disequilibrium are influences of genes on different phenotypes that may be transmitted to the next generation.

The interdependence of traits can be studied using the methods of quantitative genetics and becomes especially clear when one tries to change the expression of a particular trait by directional selection. As a rule, artificial selection changes not only the trait chosen as the criterion of selection. An array of other traits will also be affected by the selection regime. Which traits will show correlated responses and to what extent cannot be deduced alone from their phenotypic correlations with the selected trait. Although phenotypic correlations may be indicative of the strengths of correlated responses (e.g., Cheverud, 1988; Falconer, 1989), this is not necessarily so and may be misleading (Willis *et al.*, 1991; Spitze *et al.*, 1991). Among other factors, the correlated responses depend on the extent to which the variation of the trait directly subjected to artificial selection and of associated traits is influenced by common (pleiotropic) genes, that is, on the degree of genetic correlations between traits. Linkage disequilibrium is often thought to be unimportant for maintaining genetic correlations in approximate equilibrium (Turelli, 1985; Hastings, 1989; Bürger, 1989). The chief cause for genetic correlation is therefore the manifold or pleiotropic action of genes (e.g., Bulmer, 1974). The degree of genetic correlation relates, though with some reservation especially when genetic correlations are low, to the proportion of genes that two traits have in common (Carey, 1988).

Negative genetic correlations indicate a trade-off between traits. An example is given in Fig. 1. Consider the trade-off in energy used for reprodution (R) and for survival (S). The fraction of energy allocated to reproduction is assumed to be genetic and also determines (by what is left) the fraction allocated to survival. For any fixed level of the total amount of energy, the genetic correlation between the traits R and S is negative (Bell and Koufopanou, 1986; van Noordwijk and de Jong, 1986; Houle, 1991; Stearns *et al.*, 1991). This genetic correlation does not mean, however, that R and S are controlled by the same genes.

The interdependence of traits, which is especially evident with quantitative traits, automatically means that natural and sexual selection do not act on single traits. Recent theoretical models and considerations of multivariate evolution have made it clear that knowledge of genetic correlations among traits is essential to understanding both the potential and constraints for phenotypic evolution (e.g., Lande, 1979, 1982; Cheverud, 1982, 1984; Maynard Smith *et al.*, 1985; Clark, 1987). The major detrimental effect of genetic correlation is to delay adaptation (e.g., Lande, 1982; Via, 1984; Via and Lande, 1985; Arnold, 1987).

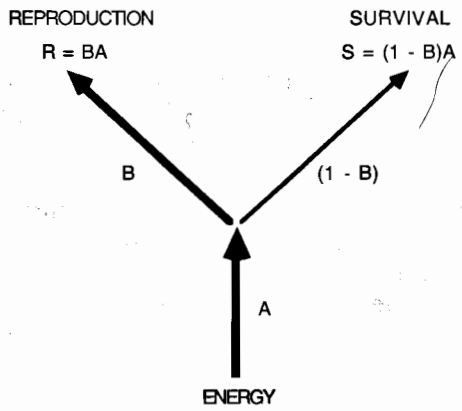

FIG. 1. The allocation of energy between reproduction (R) and survivial (S). A stands for the total amount of energy required (A = R + S), and B is the fraction of energy allocated to R. The trade-off in energy used for reproduction and survival results in a negative genetic correlation between the traits reproduction and survival. Adapted from Stearns *et al.* (1991).

An important application of the quantification of genetic correlations is the identification of developmentally or functionally integrated traits (Stearns *et al.*, 1991). There is a growing body of data concerning genetic correlations within suites of traits at various levels or combinations of levels: biochemical traits (Clark, 1990), life-history traits (e.g., Service and Rose, 1985; Dingle *et al.*, 1988; Scheiner *et al.*, 1989; Snyder, 1991), morphological traits (Atchley *et al.*, 1982; Lavin and McPhail, 1987) and their development (e.g., Cheverud, 1982; Atchley, 1984), behavioral traits (Arnold, 1981; Via, 1984; Gromko and Newport, 1988; Stevens, 1989), integration of behavioral and morphological traits (Brodie, 1989, 1992), integration of behavioral and physiological traits (e.g., Garland, 1988), integration of behavioral and neuroanatomical traits (e.g., Crusio *et al.*, 1989), integration of behavioral, physiological, and morphological traits (Fairbairn and Roff, 1990), integration of behavioral, life-history, and morphological traits (Palmer and Dingle, 1989), integration of behavioral, life-history, and physiological traits (e.g., Lynch and Roberts, 1984; Sulzback and Lynch, 1984), and the integration of behavioral and life-history traits (Gromko *et al.*, 1991). These studies have greatly increased our understanding of multivariate evolution. The application of genetic correlations is largely unexplored in causal studies of behavior (but see, e.g., Lynch and Roberts, 1984; Sulzbach and Lynch, 1984; Garland, 1988; Crusio *et al.*, 1989; Fairbairn and Roff, 1990), in which genetic correlations

may be used as tools in studying the physiological and neurobiological control of behavior.

In the following section I present the results of a behavior-genetic study in sticklebacks that was set up to quantify the genetic variance–covariance structure of different forms of aggressiveness. The results suggest the integration of a complex suite of behavioral, life-history, and endocrinological traits. The genetic variance–covariance structure of this complex of traits augments the insight into a motivational system. Additionally, evolutionary implications with respect to stickleback aggression can be deduced. Quantitative genetic methods are potentially powerful in studying the interplay of causal, functional, and evolutionary aspects of behavior.

II. Why Study Stickleback Aggression?

Among the stickleback species, male three-spined sticklebacks (*Gasterosteus aculeatus*) have the highest levels of aggression, the most pronounced breeding coloration, and the best-developed morphological defense mechanism against vertebrate predators (Bell and Foster, 1993). It has been suggested that the relative freedom from predators has facilitated the change from breeding in areas of dense vegetation to the open (Morris, 1958; Wilz, 1971; Wootton, 1976, 1984), where competition for females would be more intense. This habitat shift may have permitted the evolution of male traits that enhance competitive abilities (e.g., high aggression levels: see the following) and attractiveness toward females (e.g., red breeding coloration: Milinski and Bakker, 1990). Furthermore, this fish species is remarkably variable for a wide array of features, including the aforementioned traits, and is actually a large complex of differentiated allopatric populations and biological species (Bell, 1984; Bell and Foster, 1993). Variation among three-spined stickleback populations is (like the exaggeration of the aforementioned male traits) often interpreted in terms of adaptation (Bell and Foster, 1993), but the genetics of most traits has not been studied.

In studying the evolution of stickleback aggression, the assessment of heritable variation in male territorial aggression would be a necessary first step, but would be of limited value in understanding its evolution because natural and sexual selection do not act on single traits (e.g., Lande, 1988). Our understanding of the evolution of territorial aggression would gain substantially by knowing the important genetic relationships between territorial aggressiveness and other traits. Through reproductive physiology, territorial aggression has obvious links with other aspects of reproductive

biology such as male courtship and coloration (e.g., Munro and Pitcher, 1983; Villars, 1983). Laboratory and field research on stickleback aggression and the situations in which it may occur has been strongly biased toward territorial aggression of reproductively active males for reasons of both conspicuousness and interest. Its occurrence among juveniles or subadults and among adult females is less well known. These other forms of aggression can be very pronounced (Bakker, 1986, 1993a; Bakker and Feuth de Bruijn, 1988) and cannot be neglected when studying the evolution of aggression in this species.

Thus, sticklebacks of both sexes show aggressive behavior in a variety of contexts. Consequently, aggression is subject to diverse selective forces. This diversity can be expected to be reflected in the underlying causal mechanisms and genetic bases of different forms of aggression, making stickleback aggressiveness a suitable example with which to study multivariate evolution. I have used multiple artificial selection experiments to evaluate the extent of common genetic control of different forms of aggression and to examine the underlying hormonal influences on aggressive behavior.

III. Life-Cycle and Aggressive Behavior of Sticklebacks

The three-spined stickleback, *Gasterosteus aculeatus* L., is a small fish (5–10 cm) that inhabits waters of the Northern Hemisphere and breeds in fresh or brackish water. In spring, male sticklebacks typically develop conspicuous nuptial coloration consisting of an orange-red throat and forebelly and blue-green eyes. Males interact aggressively while establishing territories in shallow water, subsequently building a tunnel-shaped nest of plant materials that they glue together with a kidney secretion. The territory and nest are vigorously defended against intruders (rival males, large juveniles, females, other fish species). Also during courtship males may show aggressive behavior against the female. Males spawn with multiple females (up to 20: Kynard, 1978; or even more: T. C. M. Bakker, unpublished data), after which they care for the eggs and young, aggressively defending them against predators, which include cannibalistic conspecifics in many cases. Males may complete several breeding cycles during the breeding season and have thus a higher potential reproductive rate than females, although these are capable of spawning several times in a single season. According to expectation (Clutton-Brock and Vincent, 1991; Clutton-Brock and Parker, 1992), males compete aggressively for the females. In contrast, females are relatively rarely observed to compete aggressively for males, except courting females late in the breeding season

(Bakker, 1993a). Aggression is not restricted to reproductively active fish; it also occurs among subadults, nonbreeding adults, and juveniles (Bakker, 1993a). Consequently, aggression appears to occur throughout the stickleback life cycle and in nearly every social context. The diversity of stickleback aggression may also be reflected in the control of different forms of aggression. Estimating the genetic correlations among the different forms of aggression would be a powerful method of tackling this issue of common causality.

IV. Choice of the Breeding Design

Because in sticklebacks, as is the case in most other organisms, inbred or otherwise genetically well-defined strains are not available, the refined behavior-genetic analyses that are feasible with, for instance, fruitflies or house mice cannot be done with this species. Information on the genetic architecture of behavioral traits in sticklebacks is therefore necessarily less detailed. However, many behavior-genetic studies on fruitflies and house mice had been started from a purely genetic interest often at the expense of their value for ethological, behavioral ecological, and evolutionary issues. Behavior-genetic studies on less standard organisms necessarily start from natural variation in behavior and are often driven by ethological, behavioral ecological, or evolutionary questions. This enhances their chances of making significant contributions in these fields. When inbred or otherwise genetically well-defined strains are not available, there are two options left for quantitative genetic studies (Falconer, 1989). One is based on the resemblance between individuals of different degrees of relationship. This is the only possible approach when the possibility of (selective) breeding is restricted or precluded, but the relationship of individuals is known. The other option is artificial directional selection, which involves starting with a heterogeneous base population and in each successive generation choosing individuals at one extreme of the distribution of phenotypic values as parents for the next generation (for more sophisticated selection designs, see, e.g., Falconer, 1989). Directional selection effects the concentration of increasing alleles for the behavioral trait in question in the line selected in the upward direction and of decreasing alleles in the one selected in the downward direction. Artificial selection provides the most unambiguous evidence for the contribution of additive genetic variation (variation of individual genetic differences that will be passed on to the offspring) to the phenotypic variation. Although selection experiments are not designed to unravel the genetic architecture in great detail, the resulting selection lines are suitable material for further genetic, ethological, ecological, or physiological studies. Additional infor-

mation can be obtained when one trait is selected in opposite directions in independent selection lines: limitations and asymmetries in two-way responses contain information about the action of natural selection (e.g., Broadhurst and Jinks, 1974; Falconer, 1989; Frankham, 1990).

The experimental methods that I used were partly dictated by the aims and the experimental animal and these methods of quantitative genetics will be treated in some detail in the following sections. In short, they consisted of a series of double, two-way selection experiments each based on the levels of particular forms of aggression. Some explanation of the terms "two-way," "double," and "series" is in place here in order to make their meanings clear. These terms will occur regularly later in the paper. By "two-way" I mean that, starting from the same base population, two independent selection lines were founded, one line for enhanced levels and one for reduced levels of a particular form of aggression. The term "double" describes a selection experiment involving two different forms of aggressiveness; one line (or pair of lines in the case of two-way selection) was selected for one form of aggressiveness and screened for another form of aggressiveness not directly selected for, while the other line was selected the other way round, thus selected for the other form of aggressiveness and the correlated response measured for the form of aggressiveness that served as the criterion of selection in the other line. The term "series" denotes that several double, two-way selection experiments were performed each with a different combination of criteria of selection. The experiments started from a natural stickleback population.

Sticklebacks are suitable study objects because they possess a suite of attributes that makes quantitative genetic studies with this species feasible; they can be kept in relatively large numbers in the laboratory under semi-natural conditions, they have a great reproductive capacity, their generation time can be reduced to about 6 months in the laboratory, they can reproduce in the laboratory year-round by appropriate manipulation of day length and temperature, their behavior can be quantified reliably, and their ethology, ecology, morphology, and endocrinology are well studied (Wootton, 1976, 1984; Bell and Foster, 1993).

V. Pros and Cons of Estimating Genetic Correlations from Selection Designs

This section is an account of methods for and pitfalls in estimating genetic correlation and is at times rather technical. Readers who are not interested in these methodological problems and details can skip the technical parts of this section without losing the thread and the essence of the chapter.

Genetic correlations can be estimated in three ways (Falconer, 1989). The first method is based on the resemblance of related individuals and is thus analogous to the estimation of heritability that is defined as the proportion of variation in a phenotypic character in a population that is due to individual genetic differences that will be inherited by the offspring (Ridley, 1993). If a set of data permits the estimation of heritability, genetic correlations may be estimated from the same data set when two or more traits are measured on each individual. Instead of computing the components of variance of one trait from an analysis of variance, the components of covariance of two traits are computed from an analysis of covariance. The offspring–parent relationship can also be used for estimating genetic correlations. This is done by computing a so-called cross-covariance obtained from the product of the value of trait x in parents and the value of trait y in offspring, or from the reciprocal situation (trait y in parents multiplied by trait x in offspring). Usually either the geometric or arithmetic mean of the two cross-covariances is considered, but a separate analysis of the two estimates of the genetic correlation based on the two cross-covariances is useful for checking possible errors of the estimates arising from similarities between relatives caused by common environments (van Noordwijk, 1984). For example, in a natural population of great tits, the genetic correlation between the size of the eggs laid by the mother and body size of her daughters is higher than when we calculate the same genetic correlation the other way round, that is, with the size of the eggs laid by the daughters and the body size of the mother. Apparently, nongenetic influences bias the former genetic correlation (van Noordwijk, 1984).

In the second method, genetic correlations are estimated from the responses to selection in a manner analogous to the estimation of realized heritability. If selection is exerted on trait x and the correlated response of trait y is measured, then the heritabilities of both traits must be known in order to compute the genetic correlation. Even if the heritability of trait y is not known, the correlated response of y gives valuable information about the maximum value of the genetic correlation and whether the genetic correlation between x and y is positive, negative, or, with some reservation, absent.

Genetic correlations can also be computed from selection experiments without knowledge of the heritabilities of the traits. In that case a so-called double selection experiment (Falconer, 1989) has to be carried out, that is, line X is selected for trait x and screened for trait y, while line Y is selected for trait y and screened for trait x. So both lines are screened for both traits, but each is selected for a different trait (Fig. 2). Then both the direct and the correlated responses of each trait can be measured and

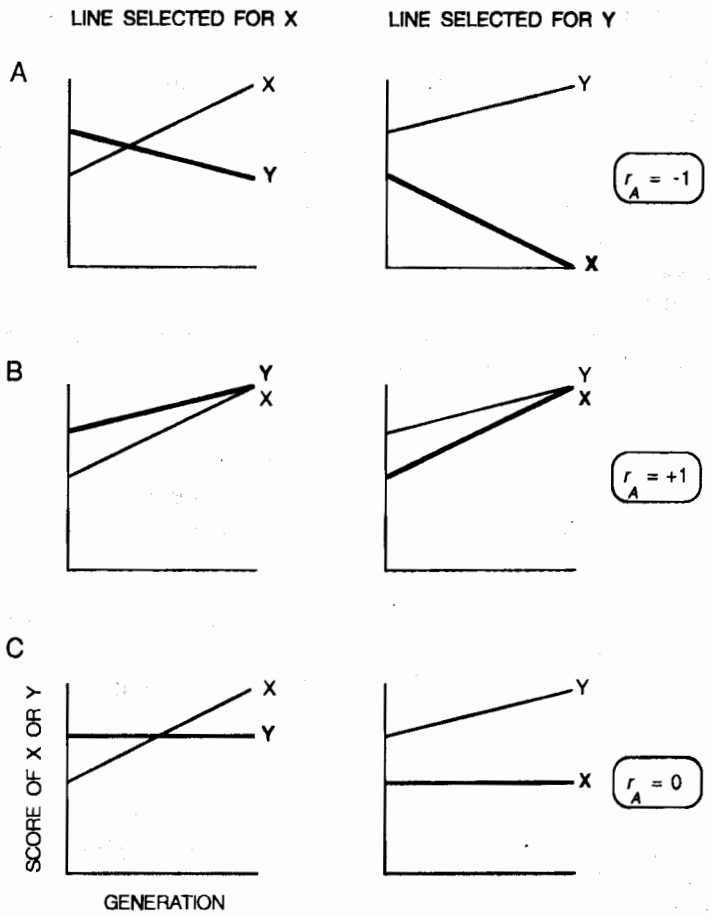

FIG. 2. Possible outcomes of a double, one-way selection experiment when there exists (A) a strong negative genetic correlation ($r_A = -1$) between the traits X and Y, (B) a strong positive genetic correlation ($r_A = +1$), and (C) when a genetic correlation between X and Y is absent ($r_A = 0$). The responses of trait X and the correlated responses (in bold) of trait Y to selection for enhanced levels of X are shown on the left side. On the right side are the responses of trait Y and the correlated responses (in bold) of trait X to selection for enhanced levels of Y.

a joint estimate of the genetic correlation obtained from the equation (Falconer, 1989)

$$r^2_A = (CR_x/R_x)\,(CR_y/R_y),$$

where r_A is the genetic correlation between traits x and y, and R symbolizes the response and CR the correlated response with subscripts x and y

according to the trait referred to. When the genetic correlation between x and y is +1 (Fig. 2B), then direct selection for x (in the line selected for x) and indirect selection for x (in the line selected for y) are equally effective in changing x. The same is true for trait y. With a genetic correlation of −1 (Fig. 2A) the magnitude of the changes in x (or y) produced by direct and indirect selection for x (or y) are the same but in opposite directions. Without genetic correlation between x and y (Fig. 2C), selection for x has no effect on y, and vice versa. Hill (1971) provides a formula for the standard error of genetic correlation that is estimated this way. Since in the formula the standard errors of the two heritabilities appear in the numerator, an experiment designed to minimize the sampling variance of heritability will also have the optimal design for the estimation of a genetic correlation.

There exists some scepticism around the estimation of genetic correlations for the following reasons. Estimates of genetic correlations are usually subject to large sampling errors and are therefore seldom very precise. Quantitative genetic studies based on the resemblance of related individuals require fairly large sample sizes relative to phenotypic studies (e.g., Klein *et al.*, 1973; Gianola, 1979; Cheverud, 1988; Falconer, 1989). This is a consequence of a two-level sampling problem. Both the number of offspring per family and the number of families are important in determining the sampling variation of genetic estimates (e.g., Falconer, 1989).

The method of two-way selection is somewhat less demanding as to the sample sizes necessary for reliable genetic estimates, because it accumulates genetic differences over a number of generations in either direction. Heritabilities that are estimated from two-way selection studies have smaller sampling variances than estimates from parent–offspring, full-sib, or half-sib analyses with the same total number of individuals recorded (Hill, 1971). The estimation of the sampling variance of heritability from selection studies is, however, not straightforward. In selection studies, realized heritability is usually estimated from the regression of cumulative response on cumulative selection differential (e.g., Falconer, 1989; for alternative estimators, see Hill, 1972a,b). The sampling variance of the regression coefficient, which had frequently been used as the sampling variance of the realized heritability, seriously underestimates the sampling variance of the realized heritability because of autocorrelation and may be one-tenth or less of the correct value (Dickerson, 1969; Hill, 1971, 1972a,b). Because in selection studies the number of selected parents is small, the variance of the population mean increases each generation as a result of genetic drift, and the generation means become correlated. In standard regression analysis the observations are assumed to have equal variance and be uncorrelated. After a few generations of selection most

variance is contributed by drift. Hill (1972a,b) gives the correct expressions (though approximations) for calculating the sampling variance of heritability with different selection designs (one-way selection with or without a control line, two-way selection, selection in one or both sexes). The best estimate of the sampling variance of heritability is the direct estimate obtained from the variance between replicate selection lines. One or a few replicates will not be sufficient for this purpose. Two-way selection is the most efficient (i.e., produces the smallest sampling variance of heritability) selection design (Hill, 1972b).

The estimation of genetic correlations from the correlated responses of traits not directly selected for may pose interpretative problems (Henderson, 1989). In two-way selection experiments, modest but significant line differences in traits not directly selected for may be due to genetic drift and thus totally irrelevant to the originally selected trait. This problem is especially relevant when new traits are being investigated in already existing selection lines (most of which show considerable inbreeding) for which replicate lines are not available. Henderson (1989) provided a helpful decision diagram for evaluating genetic correlations between selected traits and correlated traits. The ideal experimental design would consist of replicate high- and low-selected lines. Consistency of direct and correlated responses between replicates would rule out the possibility that the responses were produced by genetic drift. An unselected control line may serve as an unselected replicate line (see the following) but is less powerful than the use of replicate high- and low-selected lines unless the unselected lines score well below or above high and low groups (Henderson, 1989). The size of the drift effect will be considerably smaller than that produced by common genetic influences when even a modest ($0.25 < r_A < 0.40$) genetic correlation exists between two traits x and y (Henderson, 1989). When replicate lines are not available, effect size can be used as a guide to interpreting whether significant high- and low-selection line differences in y are likely to be due to drift or pleiotropy (Henderson, 1989).

An experimental design consisting of double selection experiments reduces the aforementioned difficulties. In double selection experiments, the responses and correlated responses serve as mutual controls for drift effects and compensate for not having replicate selection lines, especially when a series of double, two-way selections are run (Bakker, 1986). The reason why such a design controls for genetic drift is the same as for replicate selection lines: consistency of direct and correlated responses in both designs reduce the probability that the observed changes are produced by chance effects. Let us consider a double, two-way selection experiment involving the traits x and y. In one pair of lines trait x serves as the criterion of selection (one line selected for high x and one for low

x) and the correlated responses of y are measured in both lines. The other pair of selection lines is selected for high y and low y, respectively, and screened for changes in x. If genetic drift always occurs but in an arbitrary direction, then, after one generation of selection, there would be a 50% chance in each of the four selection lines of x and y changing in the same direction, that is of high x going with high y, or low x with low y. Since we have four independent selection lines, there would be a $(0.5)^4 \times 100\% = 6.25\%$ chance of a "perfect" association in all four lines by chance alone (i.e., always the appropriate association of high and low x's and y's). Probabilities are of course much less when observations are made over more than one generation. Probabilities are further reduced when we consider a series of double, two-way selection experiments, that is, they involve more lines selected for different traits. Including a third pair of selection lines for, say, trait z, and screened for the other two traits x and y (the lines selected for x and y should then also be screened for z), would make genetic drift a very unlikely cause for consistent direct and correlated responses. This is in essence the design that I applied in studying common genetic control of different forms of aggression in sticklebacks.

The potential problems and difficulties in estimating genetic variances and covariances may be a considerable obstacle to initiating quantitative genetic studies of behavior with organisms that are not commonly used in genetics. This constraint becomes especially clear in studies on sexual selection through female choice. There exist a plethora of population genetic models for the evolution of male sexual ornaments through female choice (e.g., Maynard Smith, 1991). In all of these models, assortative mating will generate a positive genetic correlation through linkage disequilibrium between male ornaments and female preference for them as long as there is genetic variation for these traits. Although ten years ago Arnold (1983) advocated the measurement of this genetic correlation and gave a guideline of how to measure it, it was only recently estimated at the within-population level (Bakker, 1993b). In cases where genetic correlations are caused by linkage disequilibrium, artificial selection may be a less appropriate method to estimate genetic covariance, unless special care is taken to maintain the genetic correlation. The estimation of genetic correlations between the sexes using artificial selection experiments has recently been debated with respect to male and female mating speeds in *Drosophila melanogaster* (Arnold and Halliday, 1992; Gromko, 1992; Butlin, 1993; Stamencovic-Radak *et al.*, 1993).

At the species level, a positive genetic correlation between senders and receivers of sexual (in particular, acoustic and chemical) signals is of interest because it produces prezygotic reproductive isolation and thus

promotes speciation. Hybrid females between closely related taxa show in many cases a mating preference for secondary sexual traits of hybrid males (e.g., Butlin and Ritchie, 1989; Boake, 1991; Ritchie, 1992). Neuroethologists have raised the possibility that this genetic correlation could result from pleiotropic effects of genes influencing the neural networks that control sending and receiving. The concept of common genetic or physiological control of these male and female behaviors has been termed genetic coupling. The evidence for genetic coupling is weak (Butlin and Ritchie, 1989), and backcrosses and recombination will be necessary to test linkage of genes.

VI. Genetic Correlations and the Causation of Aggressive Behavior: Double Selection Experiments

A. Methods

1. Experimental Design

To start the selection experiments with a genetically heterogeneous population, the base population was derived from laboratory-bred progeny of a large sample of parents (25 mating pairs) collected from a freshwater stickleback population (near Vaassen, Netherlands). I started independent selection lines for each of three forms of aggression each of which were selected for enhanced and reduced levels of aggression. In each generation about three parental pairs with extreme levels of aggression were selected to propagate the selection lines. In each line and in every generation about 15 progeny of either sex were tested for their aggression levels. Five generations (the base population and four selected generations) were involved.

There are several possibilities for a control line in the analysis of direct and correlated responses to selection (e.g., Falconer, 1989; Gromko *et al.*, 1991): (a) paired high and low lines may be used as controls for one another; (b) an unselected line may be maintained at the same effective population size as the selection lines (an inbred control); and (c) an unselected line may be maintained at a larger effective population size than the selection lines (an outbred control). I used a combination of options (a) and (c), because I was interested in the direct and correlated responses to selection for enhanced and reduced levels of aggression. Since the paired high and low lines were propagated from about the same number of parents, they provided a control for inbreeding depression. Consistent differences between paired high and low lines are most likely to be due

to selection, regardless of whether selection has produced inbreeding depression or not.

Responses to two-way selection for fitness traits are expected to be asymmetrical, with greater responses in the direction of lower fitness (e.g., Falconer, 1989; Frankham, 1990). Therefore, in addition to the two-way (also called bidirectional or divergent) selection lines, a separate control line was maintained that was propagated by about 10 different parental pairs. The use of such an outbred control line allows the analysis of asymmetry. The control line was maintained throughout the generations of selection and used partly to detect environmental deviations and partly for assessing responses and correlated responses in the separate high and low lines. For these purposes it was not necessary (though it would have been better) to screen the control animals in every generation; the control line was measured at generations 0 and 2, and average control line levels were calculated (Bakker, 1986).

A further extension of the experimental design involved the screening of the fish in every generation and in each line for all the investigated forms of aggression. The study thus consisted of a series of double selection experiments permitting the estimation of genetic correlations among different forms of aggression.

For practical reasons I had to refrain from the use of replicates, but the design of double selection experiments compensates for not having such replicate selection lines (see foregoing). This is especially true when the same traits are screened in several different two-way selection lines. Although inbreeding was avoided as much as possible, in later generations some inbreeding could not be avoided (generation 1, no inbreeding; range of coefficients of inbreeding among lines in generation 3, 0.12–0.25; Bakker, 1986). Further details on experimental design can be found in Bakker (1986).

2. *Behavioral Tests and Selection Lines*

To standardize rearing conditions, that is, to exclude paternal effects, clutches of eggs were transferred to an artificial hatching system shortly after fertilization. Juveniles used to establish each generation were isolated well before the onset of juvenile aggression in small (10 liter) tanks. Several forms of aggressiveness were quantified in standardized aggression tests (van Iersel, 1958). In these tests, the fish were offered an appropriate opponent in a glass tube inside their tanks or in a polyacrylic plastic chamber hung on the front of their tanks. When the fish reached the opponent, the duration of the aggressive acts of biting and bumping at the opponent was scored during 5 min.

During the juvenile stage the aggressiveness of juvenile males and females toward a juvenile opponent was screened (juvenile aggressiveness).

Adult females were presented with a subadult (female aggressiveness). The aggressiveness of reproductive, territorial males was measured toward a rival male (territorial aggressiveness) and toward a ripe female (courtship aggressiveness). Additionally, reproductive males were tested for their dominance ability. If two reproductive, isolated males are simultaneously introduced into a tank unfamiliar to both and just large enough for the settlement of one territory, then one of the males usually dominates the other after a short and intense fight (Bakker and Sevenster, 1983). The dominant male begins nest building, while the inferior male remains quiet at the water surface or hidden between plants where he is attacked by the dominant male if he moves. Dominance ability was measured by making all pairwise comparisons of relative dominance among a group of about 15 individually isolated males. The males can then be arranged in a linear order of dominance based on the probability of winning the dominance contests (Bakker and Sevenster, 1983; Bakker, 1985, 1986).

Before starting the selection experiment, I measured the consistency of aggressive behavior in each of the test situations by calculating repeatabilities (Falconer, 1989). The levels of aggression in each situation were significantly repeatable (Bakker, 1986). Because I used the mean of several aggression tests per individual as the aggression score in the selection experiments, the repeatability of these scores would have been even higher (Falconer, 1989).

Independent selection lines, one each for enhanced and reduced levels of aggression, were established for each of three forms of aggression. Juvenile aggressiveness in juveniles of both sexes served as the criterion of selection in founding lines with high (JH) and low (JL) levels of juvenile aggression. Similarly, territorial aggressiveness of adult males and female aggressiveness of adult females were used as the criteria of selection in the high (TH) and low (TL) territorial aggression lines. In establishing the high (DH) and low (DL) dominance lines, the male's dominance ability was used as the criterion of selection. In addition to these six selection lines, an unselected control (C) line was maintained by breeding randomly selected adults.

In the juvenile and territorial aggression lines both sexes were selected. Though facilitating quick responses to selection, this procedure complicated the analyses of the selection lines (details in Bakker, 1986).

B. Direct and Correlated Responses to Selection

1. Direct Responses

Selection for reduced juvenile aggressiveness produced significant divergence from the control line in both sexes after one generation of selection. The differences increased in the ensuing two generations (Figs. 3A

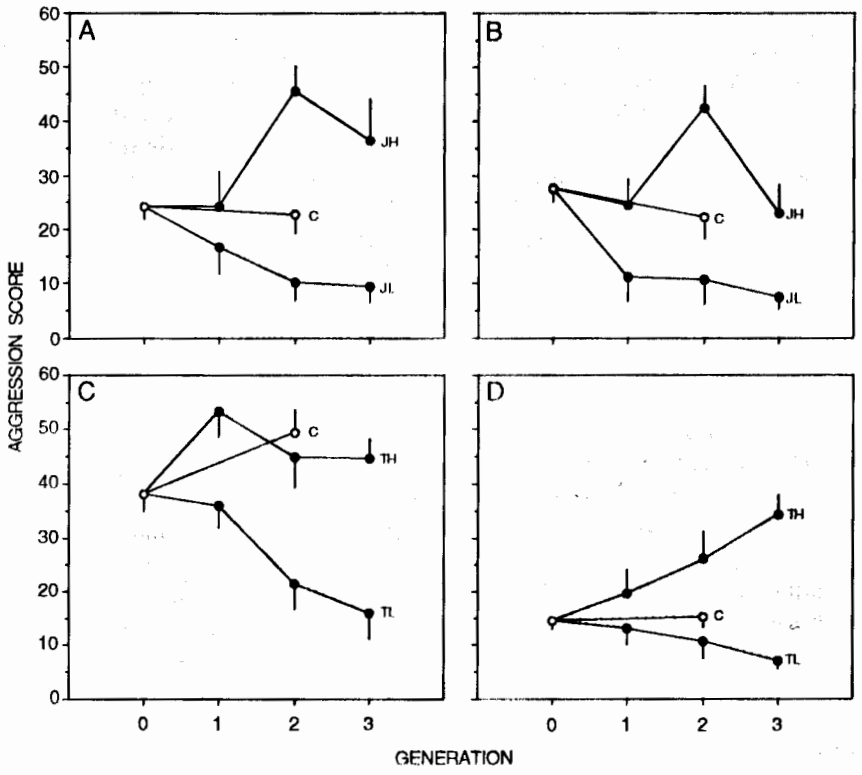

FIG. 3. Responses to three generations of selection (solid circles) for high and low levels of juvenile (JH and JL lines) and territorial (TH and TL lines) aggression. Aggressiveness in an unselected control line (C) is indicated in generations 0 and 2 (open circles). Juvenile aggressiveness was measured and selected for in juvenile (A) males and (B) females. Territorial aggressiveness was measured and selected for in reproductive (C) males and (D) females. Aggressiveness is expressed as the mean percentage of biting and bumping time against an opponent during weekly 5-min standardized aggression tests. Error bars represent one standard error of the generation mean. Adapted from Bakker (1993a) by permission of Oxford University Press.

and 3B; Bakker, 1986). Selection for enhanced juvenile aggressiveness was less successful, producing significant divergence from the control line only in the second generation. In the third, levels of aggression were similar to those in the control line in the second generation. Similarly, selection for reduced territorial aggression produced significant divergence from the control line in reproductive males, but selection for enhanced aggression did not (Fig. 3C; Bakker, 1986). In the females, however, selection in both low and high territorial aggression lines produced signifi-

cant differences from the control line by the third generation of selection (Fig. 3D; Bakker, 1986). So significant responses were obtained after a few generations of selection for reduced levels of juvenile aggression in both sexes, reduced levels of male territorial aggression, and reduced and enhanced levels of female aggression. The levels of aggression in the control line were in general rather constant, indicating limited environmental changes during the period of selection (Fig. 3). Territorial aggressiveness of control line males was probably underestimated in the base population (Fig. 3C) because of some slight methodological differences between aggression tests in generation 0 and later generations (Bakker, 1986).

Because the standard errors of realized heritabilities in Bakker (1986) were estimated from the regression analyses, I reanalyzed the data to estimate correct standard errors according to expressions given in Hill (1972a,b). Furthermore, I used a square-root transformation of the aggression scores (increased by 0.5) of adult females to meet the normality assumptions of the analysis. Realized heritabilities were estimated from the regression of cumulative response on cumulative weighted selection differential (Table 1); I have not included estimates for the high juvenile aggression line because of the apparently nonlinear response in both juvenile males (Fig. 3A) and females (Fig. 3B). The combined two-way responses yielded estimates that ranged from 0.23 to 0.37. These values agree with h^2 estimates for aggressiveness in other species (reviewed in Bakker, 1986) and lie around the mean value for behavioral traits in general (Mousseau and Roff, 1987). Because of asymmetry in two-way response the estimates from the single selection lines had a much wider range (0–0.64).

Analysis of dominance abilities was less straightforward because dominance had to be measured in contests between two individual males. The outcome of any contest therefore depends on the phenotypes of both males. In each generation, the joint response to two-way selection for dominance was determined from interline dominance tests with males randomly chosen from both lines. Selection for low and high dominance ability produced significant divergence between the two lines by the third generation (Fig. 4), at which time males from the high dominance line dominated males from the low dominance line in 19 out of 24 dominance tests ($\chi^2 = 8.17$, $P < 0.01$; Bakker, 1986). In the second selected generation, males from the high dominance line won 5 out of 10 contests against control males, while males from the low dominance line won only 3 out of 10 contests. This nonsignificant trend was confirmed by the results of dominance tests between both dominance lines and the other selection lines in the third generation (see the following) suggesting that the divergence in the high and low dominance lines was due to a decrease in the dominance ability of males from the low dominance line rather than an

TABLE I
REALIZED HERITABILITIES (h^2) FOR DIFFERENT FORMS OF AGGRESSIVENESS[a]

Type of selection	$h^2 \pm SE^c$	F^d	P^d
JL males	0.51 ± 0.28	43.17	<0.012
JH + JL males	0.37 ± 0.19	46.76	<0.011
JL females	0.64 ± 0.28	73.51	<0.007
JH + JL females	0.25 ± 0.14	1.73	>0.15
JL males + females	0.57 ± 0.20	80.76	<0.007
TH males	−0.01 ± 0.11	0.01	>0.46
TL males	0.58 ± 0.18	592.83	<0.001
TH + TL males	0.23 ± 0.11	21.07	<0.023
TH females[b]	0.34 ± 0.12	164.96	<0.003
TL females[b]	0.27 ± 0.15	4403.30	<0.001
TH + TL females[b]	0.31 ± 0.12	422.98	<0.002
DH + DL males	0.34[e]		

[a] Estimations from the regression of the selection response on the cumulative selection differential of the various selection lines. SE, standard error; *F*, variance ratio; *P*, one-tailed probability; 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.

[b] Aggression scores (increased by 0.5), square-root-transformed.

[c] SE calculated according to Hill (1972a,b).

[d] *F* and *P* from regression analysis.

[e] Approximation based on ranks (see Bakker, 1986).

increase in that of males from the high dominance line. The ranking scale of dominance ability does not allow the formal estimation of heritability; an approximation based on ranks (see Bakker, 1986) was calculated and this fell within the range of heritabilities of other forms of aggressiveness (Table I).

These results demonstrate that there is heritable variation for each of different forms of aggressiveness. The apparent lack of response of males to selection for enhanced territorial aggressiveness (Fig. 3C) and dominance ability (Fig. 4) can probably best be explained as a consequence of long-term selection for high levels of territorial aggression and dominance ability in the natural population (Bakker, 1986). An obvious relationship between aggressiveness and fitness operates via territory size of reproductive stickleback males. In homogeneous habitats, males with large territories initiated more attacks toward rivals (van den Assem, 1967; Black, 1971) and experienced superior reproductive success through enhanced courtship success and enhanced parental success (less often victim of

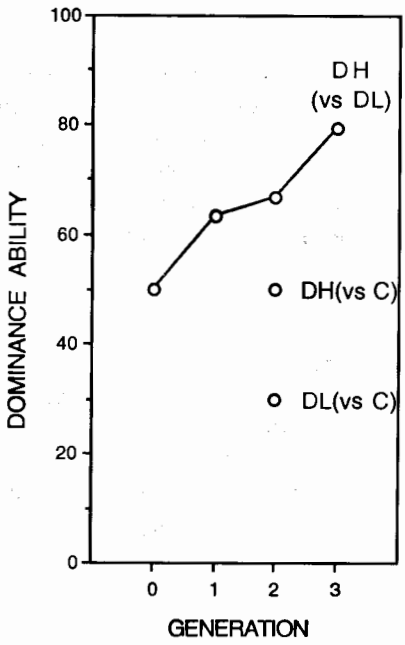

FIG. 4. The response to three generations of selection for high (DH line) and low (DL line) dominance ability. Dominance ability in each generation was measured as the proportion of cases in which reproductive males from the high dominance line won dominance contests against males from the low dominance line in a small tank unfamiliar to both males (including the first unselected generation). Males from the high and low dominance lines were also scored for dominance abilities against the control line (C) in the second selected generation. Adapted from Bakker (1993a) by permission of Oxford University Press.

sneakers and less disturbed paternal care) both under seminatural conditions in the laboratory (van den Assem, 1967) and under field conditions (Goldschmidt and Bakker, 1990). In contrast, the apparent lack of response to selection for increased levels of juvenile aggression in both males and females (Figs. 3A and 3B) may result from reduced embryonic viability and correlated increases in female aggressiveness during courtship that reduce the probability of successful spawning. Both were observed in the high juvenile aggression line (Bakker, 1986). Moreover, juvenile aggressiveness, male territorial aggressiveness, and male dominance ability were each significantly greater in laboratory-bred offspring from wild-caught parents originating from the population used in the selection study than they were in offspring from an allopatric Dutch population (Bakker, 1993a). This result may suggest that natural and sexual selection have favored high aggression levels in the population used in the selection experiments.

2. *Correlated Responses*

The fish in each selection line and in the control line were screened for all the investigated forms of aggression. The experimental design consisted in fact of a series of double selection experiments permitting estimation of genetic correlations (Falconer, 1989). Thus, each female in each line was assayed for both juvenile and female aggressiveness. Males were assayed for juvenile aggressiveness, territorial aggressiveness, dominance ability, and courtship aggressiveness. From these data, the mean score for each form of aggression was calculated for each generation in each line. A single mean for each form of aggression was calculated for each sex in the control line. The selection lines were then compared pairwise for two forms of aggression; one form of aggression had been directly selected for in one line, and the other form had been used as a criterion of selection in the second line. Thus, for example, as a direct response to selection, mean male territorial aggression was determined for each of three generations of the low territorial aggression line. Three mean scores were also calculated for the correlated response of juvenile aggressiveness in the same line so that the correlation between the two forms of aggression could be examined. The same comparison was made for the reverse case, that is, between the direct response of juvenile aggressiveness in males of the low juvenile aggression line and the correlated response of territorial aggressiveness in the same line. We then have the same situation as was visualized in Fig. 2. When we combine the left and right graphs of Fig. 2 and plot trait x on say the x axis and trait y on the y axis, we can calculate two regression lines, one for each selection line. For selection line X the slope equals (correlated response of y)/(response of x), whereas for line Y this is (response of y)/(correlated response of x). From the theory of quantitative genetics (e.g., Falconer, 1989) it can be deduced that the more the slopes of the two regression lines differ from each other the smaller is the genetic correlation between x and y; the regression coefficients are equal and positive when $r_A = +1$, equal and negative when $r_A = -1$, and maximally different when $r_A = 0$.

Plotting the relationships between the direct and correlated responses of two forms of aggression in a double selection experiment gives us a first impression of the genetic relationships between different forms of aggression. The advantage of this method is that all lines can be included, thus also the ones that did not show significant heritability (namely, the high juvenile aggression line and high territorial aggression line). The graphs give us an impression of how closely the direct and correlated responses were matched among the generations of selection, and of the direction and extent of the genetic correlations. The estimation of genetic

correlation only makes sense with selection experiments that demonstrate significant heritability. I therefore used only the low selection lines in estimating genetic correlations.

The genetic relationship between juvenile aggressiveness in females and adult female aggressiveness was assessed using the means of the control line, high and low juvenile aggression lines, and high and low territorial aggression lines. The regression of juvenile aggression on female aggression was similar among the generation means of the juvenile aggression lines and those of the territorial aggression lines (Fig. 5A), despite the nonlinear response in females of the high juvenile aggression line (Fig. 3B). This indicates that the direct and correlated responses of both juvenile and female aggression were about equally strong, suggesting that the same loci affect both forms of aggression. The genetic correlation between female aggressiveness and juvenile aggressiveness was calculated using the most reliable data: because of the nonlinearity of response and correlated response in the high juvenile aggression line, only the data from the low juvenile aggression line and low territorial aggression line were used. The genetic correlation was estimated according to Falconer (1989) from the mean responses and correlated responses per generation (calculated from the regression of mean aggression on generations of selection; Nagai *et al.*, 1978) and amounted to 1.05 (Table II). The standard error of 0.12 is probably underestimated because the genetic correlation is close to one (Hill, 1971). In contrast, the correlated responses of both juvenile and territorial aggression in males were less strong than were the direct responses of these two forms of aggression (Fig. 5B), suggesting that in males juvenile aggressiveness is only partly governed by the same genetic factors as territorial aggressiveness. The genetic correlation between juvenile aggression and territorial aggression in males was calculated using the mean responses and correlated responses per generation in the low juvenile aggression line and low territorial aggression line, and was 0.50 (Table II). The significant positive correlation between territorial aggression (direct responses) and courtship aggression (correlated responses) among generation means of the high and low territorial aggression lines (Fig. 5C) suggests a positive genetic correlation between these two forms of aggression and may point to common genetic influences between these two forms of aggression (see also Sevenster, 1961).

Selection for dominance ability produced little correlated change in other forms of aggressiveness; fish (males and females) from the high and low dominance lines did not differ significantly in any of the other forms of aggressiveness (males: juvenile, territorial, and courtship aggressiveness; females: juvenile and female aggressiveness) (Bakker, 1986). This suggests that male dominance ability is affected by genetic factors different from

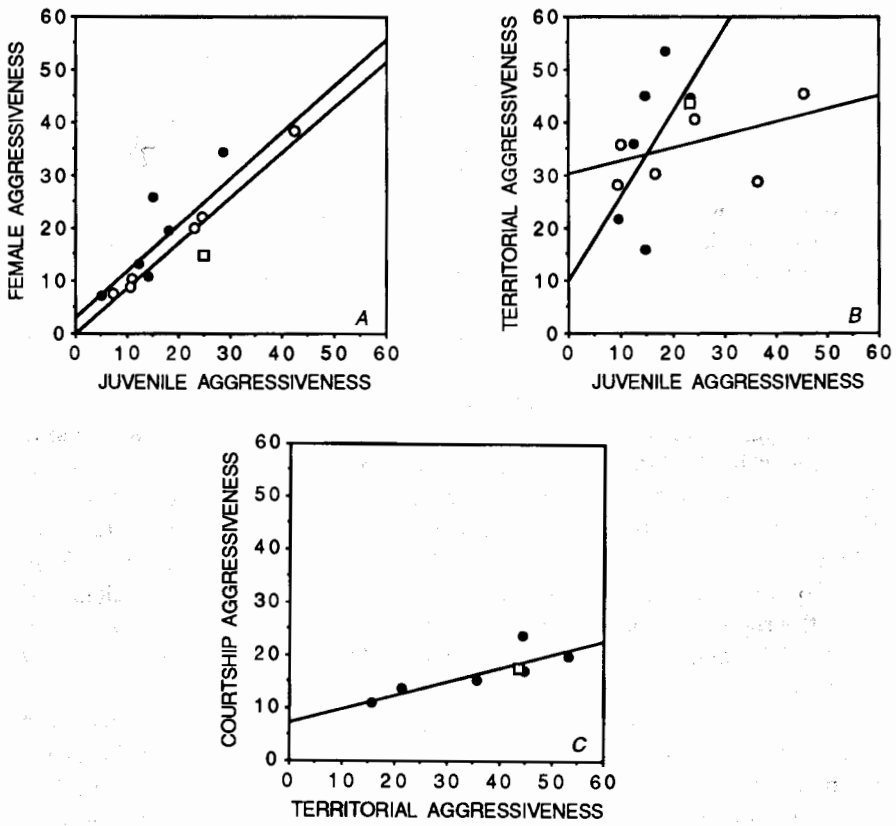

FIG. 5. Phenotypic correlations between (A) juvenile and female aggressiveness, (B) juvenile and male territorial aggressiveness, and (C) territorial and courtship aggressiveness. The data points (circles) on each graph represent the means of the aggression scores of all individuals in a single generation in a selected line. A single mean was calculated for the control line (open squares) using scores of individuals in the base population and in the second generation of the control line. (A) Relationship between aggression of juvenile and adult females in three generations of lines selected for high and low juvenile aggression (open circles; $y = -0.151 + 0.860x, r^2 = 0.93, F = 65.90$, d.f. $= 1,5, P < 0.001$, 2-tailed) and for high and low territorial aggression (solid circles; $y = 2.293 + 0.880x, r^2 = 0.54$, $F = 5.80$, d.f. $= 1,5, P = 0.06$). (B) Relationship between aggression of juvenile males and territorial aggression of reproductive males in three generations of lines selected for high and low juvenile aggression (open circles; $Y = 30.333 + 0.246x, r^2 = 0.21$, $F = 1.32$, d.f. $= 1,5$, $P = 0.30$) and for high and low territorial aggression (solid circles; $y = 10.146 + 1.597x, r^2 = 0.39, F = 3.17$, d.f. $= 1,5, P = 0.14$). (C) Relationship between territorial and courtship aggression of reproductive males in three generations of lines selected for high and low territorial aggression (solid circles; $y = 7.253 + 0.259x, r^2 = 0.70$, $F = 11.57$, d.f. $= 1,5$, $P = 0.02$). Adapted from Bakker (1993a) by permission of Oxford University Press.

TABLE II
REALIZED GENETIC CORRELATIONS (r_A) BETWEEN DIFFERENT FORMS OF AGGRESSIVENESS[a]

Form of aggression	Direct response	Correlated response	$r_A \pm SE^c$
Females			
Juvenile aggression	−5.27	−5.73	1.05 ± 0.12
Female aggression[b]	−0.36	−0.36	
Males			
Juvenile aggression	−4.90	−2.90	0.50 ± 0.26
Territorial aggression	−9.81	−4.07	

[a] Realized genetic correlations were estimated according to Falconer (1989) from the mean direct and correlated responses per generation of males and females in the low juvenile and low territorial aggression lines.
[b] Aggression scores (increased by 0.5), square-root-transformed.
[c] SE was calculated according to Hill (1971).

those affecting juvenile, territorial, or courtship aggressiveness. The apparent absence of genetic correlation between juvenile aggressiveness and dominance ability was further substantiated by the outcomes of interline dominance tests in the third generation of selection. Males from the high and low juvenile aggression lines had dominance abilities similar to males from the high dominance line (Bakker, 1986). Because a genetic correlation between juvenile aggressiveness and dominance ability was not detectable in males of four selection lines (high and low juvenile aggression lines, and high and low dominance lines), and because selection for dominance in males did not lead to significant changes in aggression scores of females of the high and low dominance line, it is unlikely that the absence of genetic correlation between the two forms of aggression was caused by genetic drift.

Two-way selection for territorial aggressiveness resulted, however, in parallel changes in dominance ability; males from the high territorial aggression line had dominance abilities that were similar to (or even higher than) the dominance abilities of males of the high dominance line, whereas the dominance abilities of males from the low territorial aggression line were similar to those of males from the low dominance line (Bakker, 1986). This seems in conflict with the apparent absence of genetic correlation between territorial aggressiveness and dominance ability when one

considers the levels of territorial aggression in the high and low dominance lines (see the foregoing). In this population, dominance ability correlated significantly positively with the degree of red breeding coloration but was not significantly correlated with territorial aggressiveness (Bakker and Sevenster, 1983; Bakker, 1986, 1993a). Selection for dominance ability may thus have directly acted on genetic determinants of brightness of red breeding coloration that do not affect territorial aggression levels. Also, in the interline comparisons of dominance ability, differences between lines in the degree of red coloration explained the greater part of the variation in dominance ability (r^2 = 0.92 and 0.76 in tests against males from the high and low dominance lines, respectively; Bakker, 1986, 1993a). The conflicting correlated responses in the territorial aggression lines and the dominance lines are counterintuitive and may suggest a different genetic causation of color changes in these lines (see the following), leading to the apparent absence of genetic correlation between territorial aggressiveness and dominance ability in the dominance lines, but a positive one in the territorial aggression lines via the association of both traits with the degree of red breeding coloration.

When interpreting the presence or absence of correlated responses one has to be aware of the pitfall of genetic drift, which may generate significant correlated responses in the absence of genetic correlations or prevent correlated responses from becoming significant in the presence of genetic correlations. In standard quantitative genetic analysis the effect of genetic drift is estimated from the consistency of correlated responses in replicate selection lines (Falconer, 1989). My choice of multiple double, bidirectional selection yielded more information than could have been obtained with the same number of tested individuals in a replicate selection design and at the same time allowed for the control of drift effects. Drift effects are, however, less obvious in this design because they become visible through the inconsistency of responses and correlated responses in the double, two-way selection lines. Thus more reasoning is required in weighing selection effects against drift effects than in a design where drift effects are made directly visible in replicate lines. The up and down selection lines showed in general consistent correlated responses. Moreover, by the use of double two-way selection, direct and correlated responses could be compared in two sets of selection lines. The general consistency of the results suggests a limited influence of genetic drift.

There is one weakness in the experimental design, namely, the application of simultaneous selection for male territorial aggressiveness and female aggressiveness in the territorial aggression lines. In view of the strong positive genetic correlation between female aggressiveness and juvenile aggressiveness (see the foregoing), some correlated responses of juvenile

aggressiveness in males of the territorial aggression lines could have been expected even if juvenile and territorial aggressiveness in males were genetically uncorrelated. Does this mean that the calculated positive genetic correlation between juvenile and territorial aggressiveness in males (Table II) is nonexistent? No, because the other changes of aggressiveness in the juvenile and territorial aggression lines seem to support a positive genetic correlation between juvenile and territorial aggressiveness in males. If juvenile and territorial aggressiveness were genetically uncorrelated, then the responses of female aggressiveness in the territorial aggression lines (only one sex would have been selected in the absence of genetic correlation between juvenile and territorial aggressiveness in males) would be half the correlated responses of female aggressiveness in the juvenile aggression lines (both sexes selected for the same trait) (but see Fig. 5A). Second, in the absence of genetic correlation between juvenile and territorial aggressiveness in males one would not expect a correlated response of territorial aggressiveness in the juvenile aggression lines (but, although not decisive, see Fig. 5B). The presence of some positive genetic correlation between juvenile and territorial aggressiveness in males therefore seems plausible, although influences of genetic drift are difficult to judge in this case.

The genetic correlations among different forms of aggression were comparable in sign and magnitude with the corresponding phenotypic correlations in the base population (Bakker, 1985, 1986) as often is the case (e.g., Cheverud, 1988; Falconer, 1989; but see Willis *et al.*, 1991). The genetic analysis suggests that stickleback aggression is characterized by a complex genetic correlation structure among different forms of aggression that may constrain the evolutionary response of specific forms of aggression. The demands made upon the levels of different forms of aggression may be quite different. The evolutionary trajectory that each form of aggressiveness follows might not only be dictated by the selection regimes acting upon the different forms of aggressiveness, but rather be a compromise imposed by the genetic relationships within this behavioral complex. Analyses of hormonal influences on stickleback aggression (see the following) suggest that the genetic correlation structure of aggression is part of a larger complex involving reproductive behaviors and several life-history characters.

C. Hormonal Influences

Before treating hormonal influences on stickleback aggression, two points should be made clear. First, I have not directly measured hormone levels, so hormonal involvement in the control of aggressiveness was

deduced from a number of correlated changes in the various selection lines combined with data from the literature and should be considered as circumstantial evidence. Second, in the interpretation of causal mechanisms, some effects may have been due to genetic drift. Because I did not directly select for the life-history and morphological traits involved, the relationships of these traits with aggressive behavior could only be deduced from their correlated responses to selection for aggressiveness. The consistent correlated responses of the up and down selection lines as compared with control line levels, and the plausible biological interpretation that emerges, give faith in a correct interpretation that may guide more detailed studies.

There existed differences in a number of life-history and morphological traits among the various lines selected for different forms of aggression. These differences suggested the involvement of two classes of hormones in the control of stickleback aggression (Bakker, 1985, 1986, 1993a). Juvenile aggressiveness was significantly negatively correlated with the age at sexual maturity among generation means of the low and high juvenile aggression lines (Bakker, 1986, 1993a). Thus, selection for juvenile aggressiveness was accompanied by a change in the age at sexual maturity, such that after three generations of selection both sexes of fish from the high juvenile aggression line matured on average about 2 weeks earlier than fish from the low juvenile aggression line (Bakker, 1986, 1993a). Additionally, after three generations of selection, the onset of juvenile aggression was on average about a week later in fish from the low juvenile aggression line as assessed in standardized groups of juveniles (Bakker, 1986). Finally, the incidence of female ripeness was significantly lower in fish from the low juvenile aggression line than it was in fish from the high juvenile aggression line (Bakker, 1986, 1993a). These results suggest that selection for juvenile aggressiveness has acted on the (effective) level of gonadotropic hormones because teleost gonadotropins induce spermatogenesis, spermiation, and testicular steroidogenesis in males and vitellogenesis, ovarian estrogen secretion, oocyte maturation, and ovulation in females (Idler and Ng, 1983; Ng and Idler, 1983). Gonadotropins are pituitary hormones, whose secretion is triggered by light (e.g., Slijkhuis, 1978; Borg *et al.*, 1987). Secretion of gonadotropins in turn stimulates the production of gonadal hormones (androgens). Under winter conditions (short photoperiod, low temperature) androgens have a positive-feedback effect on gonadotropin synthesis (Borg *et al.*, 1986). During the breeding season (long photoperiod, relatively high temperatures) androgens inhibit gonadotropic cells (Borg *et al.*, 1985).

Attainment of sexual maturity was delayed in the low juvenile aggression line, suggesting that selection on this aspect of life history could produce

a correlated response in juvenile aggressiveness (and correlated forms of aggression). Similarly, selection on body size could indirectly influence juvenile aggressiveness because growth slows after attainment of sexual maturity (e.g., Wootton, 1976, 1984), and later-maturing individuals tend to be larger.

Additionally, females in the high juvenile aggression line appeared to mature clutches more rapidly than did females in the low juvenile aggression line. This could indicate a positive genetic correlation between aggression and clutch maturation rate, or could simply result from smaller females producing smaller clutches at a higher frequency than larger females (but see Wootton, 1973). Data from the high and low juvenile aggression lines, and control line in the second selected generation where differences among lines in the level of juvenile aggression were greatest (Fig. 3), offered some support for the former explanation (Bakker, 1993a).

Selection on territorial aggressiveness has apparently affected the level of androgen production rather than the level of gonadotropin production. Males from the high territorial aggression line had significantly enlarged kidneys (corrected for differences in body size) relative to control line males, a condition indicative of elevated levels of androgen production (Wai and Hoar, 1963; Mourier, 1972; de Ruiter and Mein, 1982), whereas males from the low territorial aggression line had significantly smaller kidneys than those of the controls (Bakker, 1986, 1993a). Parallel changes in the degree of red breeding coloration in males from the high and low territorial aggression lines (Bakker, 1986, 1993a) further support this hypothesis.

Although genetic correlations between aggressiveness and most aspects of reproductive behavior have yet to be investigated, their common hormonal control and signaling system (red nuptial coloration of the male; Bakker, 1993a) make it likely that some aspects of aggressive and reproductive behavior will prove to have evolved in concert. The probable hormonal cause of such character correlations are the androgens, which have been implicated as determinants of aggressiveness, nest building, nest-directed activities, courtship behavior, and the secondary sexual characteristics of the male (see references in Bakker, 1993a). Thus, androgen levels, levels of some forms of aggression, nuptial coloration, and reproductive behavior tend to covary over the life cycle of the male. It follows from this that differences in androgen levels among individuals or populations can lead to positive correlations between these characters (Rowland, 1984; Giles and Huntingford, 1985; McLennan and McPhail, 1989).

The interrelationships among the pituitary–gonadal axis, the aggressive system, the sexual system, and the breeding coloration of the male three-

spined stickleback in the sexual (empty nest) phase are summarized in Fig. 6. The proximate model in Fig. 6 is based on experimental data from the stickleback literature combined with the findings of this study. The model shows only those relationships that have been investigated in sticklebacks and is a simplification compared with possible relationships known from studies in other teleost fishes. For example, the influence of hormones on behavior is not one- but two-sided, that is, there is a feedback from behavior to hormone levels (e.g., Munro and Pitcher, 1983; Villars, 1983). Long daylength triggers the production of gonadotropins in the pituitary, which in turn stimulates the production of androgens in the testes. During the breeding season there is a negative feedback of androgens on gonadotropin synthesis. Both hormones of the pituitary–gonadal axis are assumed to have a positive effect on the aggressive system, which is activated by stimuli that threaten a male's resources, in the case considered in Fig. 6 a male's territory and nest. Every conspecific intruder in a male's territory

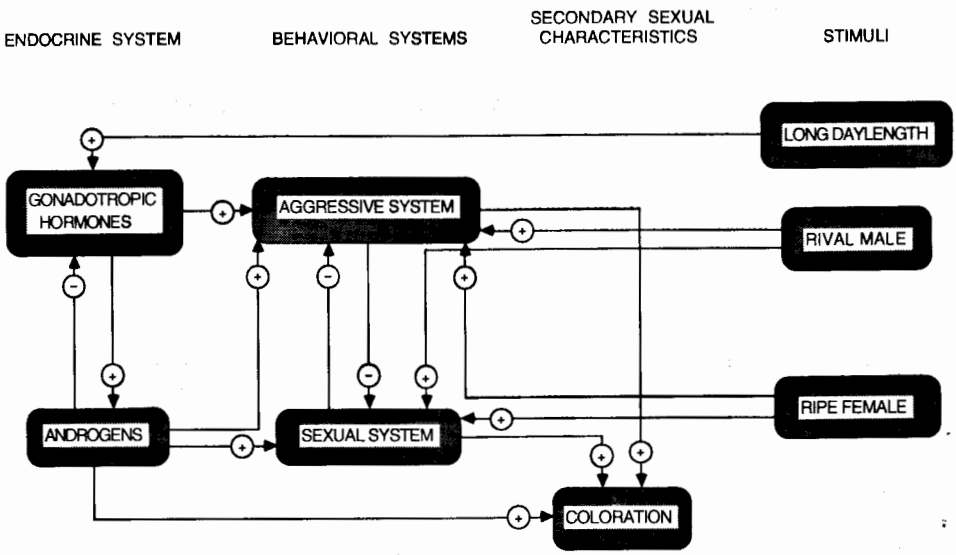

FIG. 6. Proximate model of the interrelationships among the pituitary–gonadal axis, the aggressive system, the sexual system, and red breeding coloration of the male three-spined stickleback in the sexual (empty nest) phase. Plus sign indicates stimulatory and minus sign inhibitory effects. The aggressive system is usually stimulated much more strongly by rival males than it is by ripe females, whereas for the sexual system the reverse holds (e.g., Sevenster, 1961). The intensification of coloration is greater after activation of the sexual system than it is after activation of the aggressive system (e.g., Bakker, 1993a). For further explanations see text.

is a potential threat and will be attacked, most vigorously in the case of rival males. Territorial aggression is much less but not absent toward ripe females (compare levels of territorial aggression and courtship aggression in Fig. 5C). A conspecific intruder may on the other hand increase a male's reproductive success and activate his sexual system. This increase in sexual activity is most evident when the appropriate stimuli are offered, that is, a female with a swollen abdomen that assumes a head-up courtship posture when attacked by the territory owner, but against male intruders some sexual activity can also be shown. Androgens but not gonadotropins are assumed to control the sexual system. Within an individual male there exists a mutually inhibitory relationship between the tendencies to show aggressive and sexual behavior preventing the simultaneous occurrence of enhanced levels of territorial aggression and enhanced levels of sexual activity. Finally, the male's red breeding coloration, an androgen-dependent secondary sexual trait, is intensified after activation of the aggressive and the sexual behavioral systems. The greater intensification after activation of the sexual system points to the role of coloration in female choice (Bakker, 1993a).

Although some forms of aggression are affected by androgen levels, gonadotropins may affect levels of most forms of aggression and exert a primary effect on some (see earlier, Fig. 6). For this reason, not all forms of aggression are positively correlated with reproductive behavior. For example, in lines selected for low levels of juvenile aggression and low levels of territorial aggression, males of the third and fourth selected generations displayed similarly low levels of aggressive activity during courtship. However, males from the low juvenile aggression line and the low territorial aggression line differed significantly in direct (courtship intensity expressed as the number if zigzags) and indirect (particular nest-directed activities) measures of sexual tendency; compared with males of the control line or the corresponding high line males, males from the low juvenile aggression line tended to display an enhanced sexual activity, whereas the sexual activity of males from the low territorial aggression line tended to be reduced (Bakker and Sevenster, 1989).

These findings and the extensive literature on the hormonal control of male aggressiveness outside and during the reproductive period (reviewed in Bakker, 1993a) support the interpretation that levels of juvenile aggression are affected primarily by gonadotropin levels, whereas territorial aggression is also affected by androgen levels (Fig. 6). Only when territorial aggression was selected against was there a parallel correlated response in sexual activity, a character known to be affected by androgen levels (see the foregoing). Because there exists a mutually inhibitory relationship between the tendency to behave aggressively toward a stimulus

and the tendency to behave sexually over a short time period and within an individual male stickleback (see references in Bakker and Sevenster, 1989; Fig. 6), a one-sided reduction of the aggression level as in males from the low juvenile aggression line causes an increase in sexual activity. Thus, although males from both selection lines were equally aggressive during courtship, the different selection regimes had opposite effects on sexual activity.

VII. Concluding Remarks

This study showed significant additive genetic variation in each of the different forms of stickleback aggressiveness. So natural and sexual selection can potentially change the levels of stickleback aggression in different social contexts, although there appeared little or no genetic variation for enhanced levels of most forms of aggression in this population. Furthermore, stickleback aggressiveness was characterized by a complex system in which different forms of aggressiveness were genetically correlated to varying degrees with each other. Natural or sexual selection pressures acting on particular forms of aggressiveness may thus have consequences for other forms of aggressiveness. For instance, selection for reduced levels of juvenile aggression will automatically result in reduced aggression levels of adult females, will also affect but to a lesser extent the level of male territorial aggression, but will have no influence on the males' dominance abilities. Because aggressiveness seemed to be genetically integrated into a complex character suite involving sexual behavior, secondary sexual traits, and several life-history characters, the same selection for reduced levels of juvenile aggression may have, at first sight unexpected, consequences for characters other than aggressive behavior such as the age at first reproduction.

Quantitative genetics thus provides us with tools that enable the study of the evolution and control of behavior. Of central importance is the estimation of genetic correlations. Knowledge of the degree and direction of genetic correlations is essential if we are to understand multivariate evolution. Knowledge of the physiological basis of genetic correlations is essential to understand the causal relationships between traits. In my study of stickleback aggressiveness the genetic correlations in the complex suite of aggression, sexual behavior, and life-history traits were partly based on the multiple influences of hormones of the pituitary–gonadal axis.

A comparison of two stickleback populations that differ in their life-history mode (migratory versus nonmigratory) may illustrate the genetic

integration of diverse traits and its evolutionary implications. Anadromous and freshwater populations should exhibit differences in life-history and behavioral characters because of the higher costs of a migratory life-style (e.g., Stearns, 1976; Roff, 1988), which leads to a so-called migratory life-history syndrome, in which the relevant life-history characters show positive genetic correlations (e.g., Dingle, 1988). Juveniles of anadromous populations often show reduced aggression levels (sticklebacks: Honma and Tamura, 1984; Bakker & Feuth-de Bruijn, 1988, unpublished data; Bakker, 1993a; salmonids: e.g., Keenleyside, 1979). Low levels of juvenile aggression may be favored because juveniles in anadromous stickleback populations migrate to the sea in large shoals (e.g., Daniel, 1985). Lines selected for enhanced and reduced levels of juvenile aggression suggested that juvenile aggressiveness was genetically correlated with the migratory-life history syndrome via gonadotropins (see foregoing). So, low levels of juvenile aggression in anadromous sticklebacks may also have been evolved as a correlated response to selection acting on life-history traits of the migratory-life history syndrome. I compared aggression levels in laboratory-bred offspring from a Dutch freshwater and anadromous population (Bakker and Feuth-de Bruijn, 1988, unpublished data; Bakker, 1993a). The laboratory-bred freshwater fish were significantly more aggressive and territorial during the juvenile stage than were the laboratory-bred anadromous fish. Sexual maturity of fish (males and females) of the anadromous population was significantly delayed, and freshwater males had slightly but significantly higher levels of territorial aggression but significantly lower levels of courtship activity. The differences in levels of territorial aggression did not appear to be attributable to differences in androgen levels as mean kidney size (corrected for body size) in the anadromous population did not differ significantly from that in the freshwater population. The marked differences in juvenile aggressiveness make it likely that differences in the level of (or sensitivity to) gonadotropins were responsible for the difference in territorial aggressiveness between the populations (see Fig. 6). The higher sexual activity of the anadromous males agrees with this interpretation (see Fig.6). These results suggest that (direct or indirect) selection for reduced or enhanced aggression in juvenile sticklebacks, or a more general difference in life-history pattern, has implications for the aggression and the sexual activity of mature fish.

VIII. Summary

Quantitative genetic methods allow us to investigate the causative associations between behavioral traits. Genetic correlations between traits

are useful but not often applied tools in studying the physiological and neurobiological control of behavior. In the methodological section, methods to estimate genetic correlations and pitfalls in their estimation are discussed. A breeding design consisting of double, bidirectional selection lines is advocated as being particularly suitable to causal and evolutionary studies of suites of behaviors. I used this design in studying the genetic and physiological control of functionally similar behaviors in sticklebacks, namely, aggressive behavior (biting and bumping) shown in various social contexts. The study suggests that stickleback aggression can be characterized by a complex genetic correlation structure among different forms of aggression that may constrain the evolutionary response of specific forms of aggression. Additionally, aggressiveness seemed to be part of a complex character suite in which different forms of aggressiveness, sexual behavior, the intensity of red breeding coloration and several life-history characters are genetically correlated to varying degrees. It is argued that the genetic correlations in this complex are partly based on the multiple influences of hormones of the pituitary–gonadal axis. Increased knowledge of the causation of stickleback aggression may lead to new insights and predictions with regard to multivariate evolution in this species.

Acknowledgments

I thank Enja Feuth-de Bruijn for kindly permitting me the use of some of our unpublished data, and Chris Boake, Susan Foster, Charles Goodnight, Manfred Milinski, Tim Mousseau, and Mike Ritchie for helpful comments on earlier versions of the manuscript. Manuscript preparation was funded by the Swiss National Science Foundation.

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