The Evolutionary Biology of the Threespine Stickleback

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Evolution of aggressive behaviour in the threespine stickleback

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Threespine stickleback, *Gasterosteus aculeatus* L., are highly polygynous; a male stickleback may collect as many as 20 clutches of eggs from different females in a single breeding cycle (e.g. Kynard 1978*a*). In addition, he may complete several breeding cycles during a single season (Wootton 1976, 1984*a*). We can therefore expect (Trivers 1972) strong competition to occur among male stickleback for access to females and females to be selective in their choice of mates.

Indeed, reproductive male stickleback compete directly for females, often interfering with one another's courtship (e.g. Ridgway and McPhail 1987; Goldschmidt and Bakker 1990). Competition for territories may also represent indirect competition for females, in that territory sites may be differently attractive to females (e.g. Kynard 1978*a*). In both instances, competition takes the form of intense aggressive interactions (Rowland Chapter 11 this volume).

In contrast, competition among females for mates is less often observed, presumably because throughout most of the reproductive season there is an ample supply of potential mates (e.g. Kynard 1978*a*). Late in the breeding season, however, simultaneous solicitation of males by multiple females is more common (Kynard 1978*a*; Borg 1985; Foster, pers. comm.), and aggressive interactions between courting females can be observed (Foster, pers. comm.).

These sexual selection processes, combined with the possibility that juvenile stickleback and adults outside the breeding season may maintain territories for feeding and/or shelter from predators (MacLean 1980; Bakker and Feuth-de Bruijn 1988), result in the occurrence of stickleback aggression in nearly every social situation. Aggression in the stickleback and the selective forces that act on it are thus diverse. This diversity can also be expected to be reflected in the underlying causal mechanisms and genetic bases of aggression. For a complete understanding of the evolution of aggression this complexity must be taken into account.

This chapter concentrates on intraspecific aggression. Antipredator tactics, including aggression directed at predators, are discussed by

Huntingford *et al.* (Chapter 10 this volume). The chapter is organized in three main parts. In the first, the forms of aggression exhibited by threespine stickleback (aggression of juveniles, aggression of adult females, territorial aggression of males, dominance of reproductive males) are described, and methods of measuring aggression are discussed. The central, second part reviews evidence for the existence of genetic variation in aggressiveness, both within and among stickleback populations. Attention is paid to genetic correlations between different forms of aggressiveness. Additionally, endocrine influences of the pituitary–gonadal axis in controlling aggressiveness in different phases in the life cycle of stickleback are reviewed, and the endocrine bases of genetic correlations between different forms of aggressiveness are discussed. The final section evaluates the evolution of stickleback aggression. Natural and sexual selection processes that act on a complex character set of different forms of aggressiveness, sexual behaviour, red breeding coloration, and several life history characters, are analysed. Topics that are discussed in the final part include the costs of aggression, aggression and reproductive success, and aggression and life history mode.

STICKLEBACK AGGRESSION: A MULTIFARIOUS PHENOMENON

The aggressive behaviour of threespine stickleback is strongly influenced by immediate context and by past experience (Rowland Chapter 11 this volume). Nevertheless, there are systematic differences in aggressive behaviour between life history stages and sexes, and between comparable life history stages of males and females across individuals and populations of threespine stickleback. The aggressive behaviour of reproductive (territorial) males is better studied than that of non-reproductive males, females, and juveniles. Territorial males are usually far more aggressive than are individuals in the other three groups (e.g. Wootton 1984; Bakker 1986).

Aggression of juveniles

Juvenile threespine stickleback typically, but not always, form large schools (Foster *et al.* 1988). Aggression among schooling juveniles has not been investigated in the field but has often been observed in the laboratory (Leiner 1929; Muckensturm 1969; Sevenster and Goyens 1975; Goyens and Sevenster 1976; Bakker 1985, 1986; Bakker and Feuth-de Bruijn 1988). Aggressive interactions first appear about 4 wk after hatching (Bakker 1986). During the first weeks after the onset of aggression, juveniles that are attacked typically flee without reciprocating. However, about 3 wk after the onset of aggression, attacked juveniles begin to counter-attack, a response that can lead to roundabout fights (rapid circling with spines erect). At approximately the same age, territorial behaviour first appears

(Bakker 1986; Bakker and Feuth-de Bruijn 1988). In the laboratory, aggression changes during the juvenile stage in a characteristic pattern that is not fully understood (Bakker 1985, 1986). Both sexes display similar levels of aggressive and territorial behaviour during the juvenile stage (Sevenster and Goyens 1975; Bakker 1985, 1986; Bakker and Feuth-de Bruijn 1988).

Aggression of adult females

Adult females typically forage in groups composed of adult females and non-reproductive males, leaving only to spawn (Keenleyside 1955; Black and Wootton 1970; Symons 1971; Kynard 1978a; FitzGerald 1983; Bentzen and McPhail 1984; pers. obs.). The aggressive behaviour of adult females in the laboratory resembles that of juveniles, typically involving direct attacks on one another (Leiner 1929, 1931; Wunder 1934; Sevenster and Goyens 1975; Li and Owings 1978a; Bakker 1985, 1986; Wootton 1985b). Females tend to be less aggressive when gravid, and as they age, aggressive interactions decline in frequency (Bakker 1986).

Although much less pronounced than in males, dominance relationships may also develop among females in groups (Leiner 1931; Wunder 1934; Wootton 1976; Li and Owings 1978a; Bakker 1986). For example, adult females from a Californian (USA) freshwater population established dominance relationships in the laboratory in which status was positively correlated with home range size (Li and Owings 1978a). Females of higher status also had greater access to males and were more effective than subordinates at disrupting courtship by other females. Although neither phenomenon has been studied in detail in the field, foraging territories are sometimes defended by females during the breeding season (MacLean 1980; Foster pers. comm.), and aggressive interactions frequently occur between females that are courting the same male in Crystal Lake, British Columbia, Canada (Foster pers. comm.). Defence of foraging territories has also been observed outside the breeding season in one Canadian lake (MacLean 1980).

Aggression of adult males

Pre-breeding aggression

During and outside the reproductive season, non-reproductive adult males participate in sexually mixed schools (e.g. Kynard 1978a; MacLean 1980). In the laboratory, their aggression resembles that of juveniles and females (e.g. Sevenster 1961; Wai and Hoar 1963; Baggerman 1966, 1968; Huntingford 1979). At the beginning of the breeding season, males leave the schools and establish a nesting territory. Males often must compete with other males for territory sites (e.g. Mori 1990b), and aggressiveness increases markedly at this time (as established in laboratory studies, e.g. Sevenster 1961; Baggerman 1966, 1968; Bakker 1986). In consequence, aggressive interactions, including biting, bumping, threatening, and round-

about fighting, are often observed. Males that are attacked by another male may counter-attack, flee, or assume threatening postures. The aggression shown by males in the period preceding nest building is often called 'pre-breeding aggression'. The ability of a male to obtain a territory in competition with other males is termed 'dominance ability'.

Territorial aggression

Once a territory is established, the male defends it vigorously against intruders ('territorial aggression'). Usually, the territory owner attacks rival males with a direct charge that ends in a bite or a bump, and roundabout fighting is rare. Similar attacks are directed at large juveniles, females, and often, other fish species. Once the nest is completed, the male typically courts females rather than attacking them, although aggressive behaviour may still break through at various points in the courtship sequence ('courtship aggression').

Breeding male threespine stickleback are well known for their high levels of aggression against territorial intruders, particularly against rival males. Among reproductive males of the Gasterosteidae, *Gasterosteus aculeatus* males achieve the highest levels of aggression (Morris 1958; Wilz 1971; Huntingford 1977; Rowland 1983a,b; FitzGerald 1983; Gaudreault and FitzGerald 1985).

Male aggressiveness changes during the reproductive cycle in a pattern thought to reflect changes in the value of the resource (territory and nest) being defended. Initially, the presence of a nest appears to increase the value of the resource. This increase is indicated by defence of a larger territory when a nest is present (Stanley and Wootton 1986), by a drop in aggression after removal of the nest (Symons 1965), and by a decrease in aggression with increasing distance from the nest (van Iersel 1953; Symons 1965; Black 1971; Huntingford 1976a,c, 1977; Rowland 1983 a,b, page 299 this volume). Acquisition of eggs also appears to enhance the value of the resource, and the level of aggression directed toward conspecific rivals continues to increase with the acquisition of additional clutches (Sargent and Gross 1986).

Territorial aggression during the parental phase

There has been considerable disagreement in the literature as to whether aggressive defence of the nest subsequently increases (Wunder 1928; Huntingford 1976a,b,c, 1977; Kynard 1978a) or decreases (van Iersel 1953; Segaar 1961; Sevenster 1961; Wootton 1971b) during the parental phase. This inconsistency seems to be due to differences in the distance from the nest at which aggression was measured in different studies and to changing spatial patterns of aggression by males as the embryos mature.

After egg acquisition, the size of the territory defended by the male decreases over the course of the parental phase (Black 1971; Kynard 1978a),

leading to a decrease in levels of aggression at distances greater than about 30 cm from the nest. During this period, however, levels of aggression remain high closer to the nest (Symons 1965; Black 1971). During the parental phase a distinction must therefore be made between the value of the nest and that of territory size. It is clear that the value of the nest increases once it contains eggs, but the advantage of maintaining a large territory that seems to be associated with intersexual selection (van den Assem 1967; Black 1971; Li and Owings 1978a; Goldschmidt and Bakker 1990) disappears once the male enters the parental phase. The decrease in territory size during the parental phase may reflect a trade-off between the increasing need to care for the embryos as they mature (Rowland page 338 this volume) and the need to defend the nest from egg raiders (Whoriskey and FitzGerald page 202; Foster page 394 this volume). Aggressiveness increases sharply after the eggs hatch (Sevenster 1961; Black 1971; Wootton 1971b; Huntingford 1976a,b,c, 1977; Kynard 1978a). This makes sense functionally, both because of the increased reproductive value of offspring, and because of the increased vulnerability of newly hatched young to predation by conspecifics (but see Worgan and FitzGerald 1981b).

The relationship between territorial aggressiveness and resource value is even more evident in the antipredator behaviour or boldness that males show towards predators of both adults and offspring (Huntingford et al. page 283 this volume). In the threespine stickleback, boldness towards a hunting pike and aggressiveness towards conspecifics covaried over the course of the breeding season, suggesting that these traits share internal causal factors (Huntingford 1976a,b,c, 1982; Giles and Huntingford 1984; Tulley and Huntingford 1988). Further evidence of this relationship was provided by Kynard (1978a), who demonstrated that the boldness of males confronted with a rival male paralleled that of males confronted with a trout (predator of the male) over the course of a brood cycle, increasing from the empty-nest stage to that in which males were defending fry.

In some studies such as Kynard's (1978a), the reproductive value of the guarded progeny has been shown to affect male risk-taking. Males with embryos were bolder when confronted with a preserved sculpin (Pressley 1981) or overhead stimulus (FitzGerald and van Havre 1985) than were those without embryos, and boldness toward a preserved sculpin was also correlated with embryo age. In contrast, Foster and Ploch (1990) detected no effect of embryos on male risk-taking, and FitzGerald and van Havre (1985) found no difference between male responses to an overhead stimulus when the males had embryos versus fry in their nests.

THE MEASUREMENT OF AGGRESSIVENESS: METHODS

Individual levels of aggression can be quantified in a number of ways, but the test developed by van Iersel (1958) is most widely used to measure

aggressive motivation of territorial male stickleback. This test employs a 'rival' male enclosed in a glass tube. He is introduced into the territory of an isolated experimental male at a specific distance from his nest for 5 min or so, and the numbers of bites and bumps directed at the intruder are counted. If appropriate opponents are used, this method can also be used to measure the aggressive motivation of territorial males during courtship (e.g. Sevenster 1961; Sevenster-Bol 1962; Bakker 1986), or of juveniles or adult females (e.g. Sevenster and Goyens 1975; Bakker 1986).

Variously simplified models, or 'dummy' males, have also been used to measure territorial aggressiveness. This method is valuable in that it permits alteration of specific features (for instance, nuptial coloration) of the stimulus male to ascertain the effect of the character on aggression elicited from the experimental male (Rowland page 298 this volume). The method can prove problematic, however, as there appears to be considerable inter-individual and interpopulation variation in responsiveness to dummies (e.g. Wootton 1971b; Baerends 1985; Rowland and Sevenster 1985).

A final method involves scoring of aggressive interactions between two territorial males separated by a glass partition (e.g. Baggerman 1966, 1968; Wootton 1971b). Although this method is relatively simple to carry out, interpretation of results can be complicated by habituation (Peeke 1969, 1983; Peeke et al. 1969, 1979; Peeke and Veno 1973, 1976; van den Assem and van der Molen 1969; Rowland 1988).

Each of these methods can produce different absolute levels of territorial aggression (Wootton 1971b, 1972b). Consequently, it is impossible to compare levels of aggression between studies that employ different methods of measuring territorial aggressiveness. Even when the same general method is used, minor methodological differences can have profound effects (but see Giles and Huntingford 1985). For example, the distance from the nest at which a male or a dummy is presented can significantly affect the level of aggression that is measured (see above). These problems make it hard to distinguish between interpopulation differences in aggressiveness and methodological effects, except when identical methods are applied to multiple populations (e.g. Huntingford 1982, but see Giles and Huntingford 1985). All of these methods can, however, be used to test for comparable changes in aggressiveness over the breeding cycle (Wootton 1971b, 1972b).

Establishing the frequency of aggressive interactions among individuals in standardized groups is an alternative method of quantifying interpopulation differences in aggressiveness when interindividual variation is not a primary concern. The method has been applied to juvenile stickleback (Goyens and Sevenster 1976; Bakker 1986; Bakker and Feuth-de Bruijn 1988) and was as successful as the standardized tube test in discriminating differences in the levels of juvenile aggression between genetically differentiated lines (Bakker 1986). It is probably most useful for measuring aggressiveness in juveniles, females, and non-reproductive males.

Unfortunately, controlled methods of measuring territorial aggressiveness have rarely been applied in the field or in naturalistic laboratory settings (exceptions: Black 1971; Kynard 1978a; Gaudreault and FitzGerald 1985). Instead, individual levels of aggression are usually deduced from observations of natural encounters among focal males and other individuals. These observations are relatively uncontrolled, and do not measure a standardized index of the male's aggressive motivation because the aggressive state of a male may be influenced by factors such as the number and quality of near neighbours or distance from the nest. Although it may be possible to examine associations between aggressiveness and reproductive success through field studies of this type, one must be cautious in drawing conclusions about the evolution of aggressiveness from such research. These concerns have been taken into account in the ensuing discussion. There obviously is a need for a general, standardized index of aggressive motivation in field studies. Other methodological concerns will be discussed as appropriate throughout.

CAUSES OF VARIATION IN AGGRESSIVENESS

As is probably the case for all behavioural traits, aggressiveness of threespine stickleback in all life history stages is influenced by immediate environmental and social conditions. In particular, the effects of the rival male's phenotype (e.g. nuptial coloration, body size, behaviour) on the aggressive behaviour of reproductively active males have been extensively investigated over the last 40 yr (reviewed by Rowland page 299 this volume). In contrast, study of the genetic bases of variation in aggressiveness has only recently been initiated. Because a knowledge of the extent of genetic variation underlying variation in aggressiveness is a prerequisite for evolutionary interpretation, I will begin by discussing the genetics of aggressive behaviour in threespine stickleback.

Genetic influences on aggressive behaviour

The presence of genetic variation within populations can be inferred by breeding and rearing individuals in the laboratory under identical conditions. The individuals can be scored for aggressive phenotypes at different stages in their development and, if appropriate experimental designs are used (e.g. Falconer 1981), heritabilities and genetic correlations can be calculated. A similar design can be used to examine differences among populations in the genetic bases of behaviour. The threespine stickleback is an excellent subject for research in behavioural genetics because many environmental sources of variation can be controlled in a laboratory setting (Bakker 1986). For example, fertilized eggs can be incubated in the laboratory to avoid the effects of differences in the quality of paternal care. Recent

research has established that there is a genetic basis for variation in aggression within and among populations of threespine stickleback.

Genetic differences among populations

Intriguing interpopulation differences in levels of aggression have been reported for juvenile and adult threespine stickleback when aggression has been assayed using standardized methods across all populations. As in most other aspects of phenotype, threespine stickleback populations appear to differ substantially in aggressive characteristics.

Territorial aggressiveness of reproductive males has been shown to vary among freshwater populations in Scotland (Huntingford 1982; Giles and Huntingford 1985; Tulley and Huntingford 1988). Additionally, aggressiveness during standardized courtship experiments differed between a marine population from Rhode Island, USA, and a freshwater population from Oxford, UK (Wilz 1973), between the sympatric Enos Lake species pair (Ridgway and McPhail 1984, 1987; McPhail page 421 this volume), and between parapatric anadromous–freshwater species pairs in British Columbia, Canada (McPhail and Hay 1983; McPhail page 408 this volume), and Washington, USA (McPhail 1969). Finally, juveniles, adult females, and males from Scottish freshwater populations differed in their boldness towards live and dummy predators in standardized laboratory tests (Huntingford 1982; Giles 1984a,b; Giles and Huntingford 1984; Huntingford and Giles 1987; Tulley and Huntingford 1987a, 1988; Huntingford et al. page 285 this volume), and this character is correlated with aggressiveness. Huntingford (1976a,b,c) postulated shared internal causal factors as an explanation for the correlation of these two aspects of behaviour, although this idea has yet to be tested directly. There is a consensus that predation has played a major role in moulding the behaviour and morphology of threespine stickleback (Reimchen Chapter 9; Huntingford et al. Chapter 10 this volume). To the extent that there exist correlations between aggressiveness and behavioural and morphological defences against predators, predation may have acted indirectly to mould the levels of aggressiveness displayed by threespine stickleback.

Suggestions concerning genetic involvement, and thus adaptive variation, can be deduced from the above-mentioned interpopulation differences. Strictly speaking, this is only justified if the populations concerned have been bred and raised under identical conditions to eliminate potential influences of environmental variation on variation in aggressiveness. Almost all the above-mentioned studies do not come up to these strict requirements and are therefore inconclusive with respect to genetic variation, although they may suggest adaptive variation in stickleback aggressiveness.

The best-documented interpopulation variation involves genetic differences in juvenile aggression. Bakker and Feuth-de Bruijn compared juvenile aggression and territoriality in laboratory-bred offspring from wild-caught

parents originating from an anadromous population (Den Helder, Netherlands) and a freshwater population (Emst, Netherlands) (Bakker *et al.* 1988; Bakker and Feuth-de Bruijn 1988, unpubl. data). Aggressive interactions among groups of five juveniles (mixed sexes) were measured during weekly 5 min observation periods until the first individual in the group reached reproductive maturity. During much of the juvenile stage, juveniles from the anadromous population were less aggressive than were those from the freshwater population (each week from 10 wk after fertilization onwards: Mann–Whitney U test, one tailed, $U \leq 104$, $Z \geq 2.898$, $P < 0.002$) (Fig. 12.1). Juveniles from the freshwater population were also more likely to establish territories than were juveniles from the anadromous population.

The progeny of both reciprocal crosses exhibited aggression levels similar to those of the freshwater population (Bakker and Feuth-de Bruijn, unpubl. data), suggesting dominance of alleles for higher aggression levels. Sustained directional selection is expected to lead to dominance of genes controlling the expression of the trait in the direction selected for (e.g. Broadhurst and Jinks 1974). Assuming that freshwater stickleback populations have been derived from anadromous or marine ancestors (Bell 1984*a*, 1988; Bell and Foster page 14 this volume), the genetic dominance of higher juvenile aggression levels is in the direction expected if selection has favoured greater aggressiveness in freshwater populations, as suggested by these studies.

Honma and Tamura (1984) qualitatively described parallel differences in aggressiveness between laboratory-bred juveniles of an anadromous and
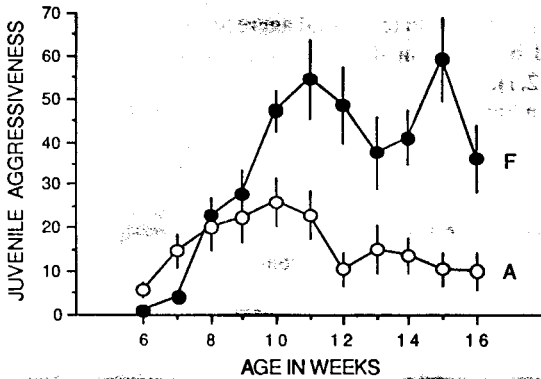

Fig. 12.1 Mean levels of juvenile aggression as a function of age (weeks after fertilization) in groups of five juveniles belonging to a Dutch freshwater (F) population (19 groups) and a Dutch anadromous (A) population (23 groups). The groups were made 3 wk after fertilization, and aggressiveness was scored as the total number of bites and bumps in the group during weekly 5 min observation periods. Error bars represent one standard error of the mean.

a freshwater population in Japan. One of the reciprocal crosses showed hybrid sterility (Honma and Tamura 1984; Honma et al. 1986).

Bakker and Feuth-de Bruijn also documented small, but significant differences in the levels of territorial aggression exhibited by laboratory-bred, isolated males from the Dutch anadromous and freshwater populations they studied (Bakker et al. 1988; Bakker and Feuth-de Bruijn, unpubl. data). Males from the anadromous population spent less time in aggressive behaviour toward an enclosed rival ($\bar{X} = 26.6$ per cent biting-bumping time during 5 min tests; $N = 44$ males) than did males from the freshwater population ($\bar{X} = 34.5$ per cent; $N = 44$ males; Mann–Whitney U test, two tailed, $U = 721.5$, $Z = 2.057$, $P < 0.04$).

Additionally, the males from these two populations differed in levels of sexual activity during tests of territorial aggression, but the relationship was the inverse of that for territorial aggression. During these tests, the number of zigzags performed by each male was recorded. The zigzag dance is a prominent component of male courtship (Rowland page 314 this volume) and is considered a reliable measure of male courtship readiness (e.g. van Iersel 1953; Sevenster 1961). Although this behaviour is displayed most often to ripe females, it is also elicited, although at a lower frequency, by male intruders. In both situations, there exists a mutually inhibitory relationship between the aggressive and sexual tendencies (Sevenster 1961; Symons 1965; Rowland 1984). Males from the anadromous population performed zigzags more often ($\bar{X} = 16.6$ per 5 min; $N = 44$) than did those from the freshwater population ($\bar{X} = 2.4$ per 5 min; $N = 44$; Mann–Whitney U test, two tailed, $U = 256.5$, $Z = 5.943$, $P < 0.001$).

Finally, the differences in territorial aggression between these populations were paralleled by apparent differences in dominance ability between the males (Table 12.1). If two reproductive, isolated males are simultaneously introduced in a tank unfamiliar to both and just large enough for the settlement of one territory, then one of the males usually dominates the

Table 12.1 Outcomes of dyadic combats between inexperienced laboratory-bred, individually isolated reproductive males from a Dutch freshwater (F) and a Dutch anadromous (A) population.

Dominance test characteristics	F dominant	A dominant	Undecided	P^{a}
Round-robin; small tanks[b]	71	27	2	<0.001
First tests[c]; small tanks	10	2	1	<0.05
First tests; larger tanks	9	1	0	<0.02

[a] x^2 test, two tailed.
[b] Tank sizes: small, 34 × 17 cm; larger, 69 × 40 cm.
[c] First tests: results are for the first tests to which each male was exposed, avoiding effects of experience (see text). Round robin: results include the outcomes of all possible pairwise combinations of males (see text).

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 upon movement.

When all pairwise comparisons of relative dominance are made among a group of individually isolated males, the males can be arranged in a linear order of dominance based on the probability of winning the dominance contests (Bakker and Sevenster 1983; Bakker 1985, 1986). Males from the freshwater population displayed greater dominance ability, regardless of whether mean dominance ability was deduced from the outcomes of all possible pairwise combinations of males or only on the basis of the first test to which each male was exposed (Table 12.1). The latter assessment avoids possible confounding effects of experience (Bakker and Sevenster 1983; Bakker et al. 1989). Population differences in territory size (e.g. Giles and Huntingford 1985) might also affect dominance ability in small (34 × 17 cm) tanks. This possibility was ruled out by assessing dominance in larger (69 × 40 cm) tanks (Table 12.1).

Genetic variation within populations

In some freshwater stickleback populations, there exists an association between morphology (lateral plate number) and aggressiveness (Huntingford 1981) or competitive abilities (Moodie 1972b; Kynard 1979a). Because lateral plate number is partly under genetic control (e.g. Hagen 1973), this association indicates, albeit indirectly, genotypic variation of aggressiveness **within** stickleback populations.

The first evidence of genetically based differences in aggression within threespine stickleback populations was provided by Goyens and Sevenster (1976), who demonstrated differences in juvenile aggressiveness between laboratory-bred progeny of different parents from a freshwater population in the Netherlands. This research stimulated more detailed and extensive studies designed to evaluate both intra- and interpopulation variation in aggression (Bakker 1985, 1986; Bakker and Feuth-de Bruijn 1988; Bakker et al. 1988; Bakker and Sevenster 1989; Bakker and Feuth-de Bruijn, unpubl. data). These studies provide the conclusive evidence for genetic variation in aggressiveness of threespine stickleback.

To assess the heritability (in the narrow sense) of aggressiveness, Bakker (1985, 1986) conducted directional selection experiments in which separate lines were artificially selected for high and low values of several forms of aggression. The base population was derived from laboratory-bred progeny of 25 males and 25 females collected in freshwater streams flowing into the Apeldoorns kanaal in the Netherlands. Independent selection lines, one each for enhanced and reduced levels of aggression, were established for each of three forms of aggression. Aggressive phenotypes of juvenile males

and females were scored, and those with the most extreme phenotypes were used to establish lines with high (JH) and low (JL) levels of juvenile aggression. Similarly, adult males were scored for territorial aggressiveness, and adult females for female aggressiveness, in establishing the high (TH) and low (TL) territorial aggression lines. In establishing the high (DH) and low (DL) dominance lines, males were scored for dominance ability and females were selected at random. In addition to these six selection lines, an unselected control (C) line was maintained by breeding randomly selected adults. The selection lines were maintained for three generations. The control line was tested against the selected lines after two generations of random mating. Juveniles used to establish each generation were isolated
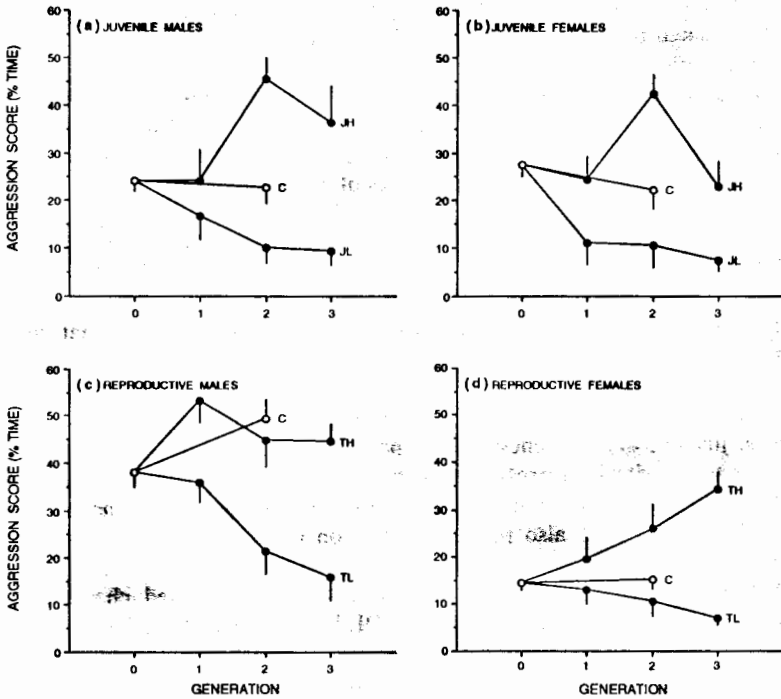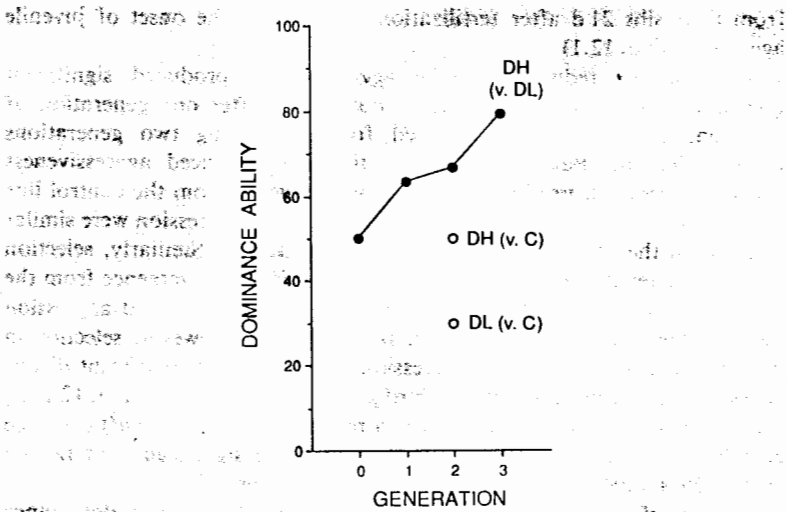

Fig. 12.2   Responses to selection for high and low levels of juvenile (JH and JL lines) and territorial (TH and TL lines) aggression. Aggressiveness in an unselected control line (C) is also presented. Juvenile aggression was measured in (a) juvenile males and (b) juvenile females. Territorial aggression was measured in (c) reproductive males and (d) reproductive females. Aggressiveness is expressed as the mean per cent biting-bumping time during weekly 5 min aggression tests. Error bars represent one standard error of the mean.

from their sibs 21 d after fertilization, well before the onset of juvenile aggression (Fig. 12.1).

Selection for reduced juvenile aggressiveness produced significant divergence from the control line in both sexes after one generation of selection. The differences persisted for the ensuing two generations (Figs. 12.2(a),(b); Bakker 1986). Selection for enhanced 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. 12.2(c); Bakker 1986). In the females, however, selection in both low and high territorial aggression lines produced significant differences from the control line by the third generation of selection (Fig. 12.2(d); Bakker 1986). In the six lines in which realized heritabilities (h^2) could be calculated for each sex (JL, TH, TL), five were significant and ranged from 0.29 ± 0.04 ($h^2 \pm$ SD) to 0.64 ± 0.07 (Bakker 1986).

Analysis of the dominance data was more complex because dominance had to be measured in contests between two individuals. The outcome of any contest therefore depends on the phenotypes of both. Within each generation of the DH and DL lines, all possible pairwise combinations of males were tested. The criterion of selection was based on the number of tests that each male had won. 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. 12.3), at which time males from the DH line won 19 of 24 dominance contests against males from the DL line ($x^2 = 8.17$, d.f. $= 1$, $P < 0.01$; Bakker 1986). In the second generation, DH males won 5 of 10 contests against control males, while the DL males won only 3 of 10 contests. This, and the results of dominance tests between both dominance lines and the other selection lines in the third generation (see below), suggested that the divergence in the DH and DL lines was due to a decrease in the dominance ability of DL males rather than an increase in that of the DH males. The estimated realized h^2 for dominance ability (combined two-way response) was 0.34 (Bakker 1986).

These results demonstrate that there is substantial heritable variation for aggressiveness in at least one population of threespine stickleback. The seeming lack of response of males to selection for enhanced dominance ability (Fig. 12.3) and territorial aggressiveness (Fig. 12.2(c)) 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). In contrast, the seeming lack of response to selection for increased levels of juvenile aggression in both males and females

Fig. 12.3   The response to selection for high (DH line) and low (DL line) dominance ability, measured as the proportion of cases in which DH males won dominance contests against DL males (including the first unselected generation). The dominance ability of DH and DL males was also scored in tests against the control line (C) in the second selected generation.

(Fig. 12.2(a),(b)) may result from reduced embryonic viability and correlated increases in female aggressiveness during courtship that reduce the probability of successful spawning. Both effects were observed in the JH line (Bakker 1986).

Genetic correlations between forms of aggression

Character correlations can profoundly affect evolutionary responses to selection, because selection on a particular trait will produce changes in correlated characters (e.g. Falconer 1981; Lande and Arnold 1983; Endler 1986). The evolutionary response to directional selection will be constrained if it produces disadvantageous changes in correlated characters because the net fitness differential may be negative. Although we certainly do not have a complete understanding of how or why different forms of aggression are correlated with one another and with other aspects of phenotype, recent research has provided some valuable insights.

Imposition of artificial selection for several forms of aggression (Bakker 1985, 1986; see above) provided evidence of genetic correlations between some but not all forms of aggression. The study consisted of a series of double selection experiments (that is, line X was selected for trait x and screened for trait y, while line Y was selected for trait y and screened for

trait x, etc.), because the fish in each line were screened for all the investigated forms of aggression. This design permitted estimation of genetic correlations (Falconer 1981), which express the extent to which the variation of two characters is influenced by common genes. One has to realize, however, that estimates of genetic correlations are subject to large sampling errors (e.g. Falconer 1981).

Each female in each line was assayed for both juvenile and female aggressiveness. Males were assayed for juvenile aggressiveness, territorial aggressiveness, dominance ability, and aggressiveness during courtship. From these data, the mean score for each form of aggression was calculated for each generation in each line. Thus, for example, as a direct response to selection, mean female aggression was determined for each of three generations of the TH 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. This was repeated in each line for all appropriate forms of aggression. A single mean for each form of aggression was calculated for each sex in the control line, using the combined scores of all individuals in generations 0 and 2 (this gave the most reliable values of control line fish in calculating genetic correlations; Bakker 1986).

The genetic relationship between juvenile aggressiveness in females and adult female aggressiveness was assessed using the control, JH, JL, TH, and TL line means (Fig. 12.4(a)). There was a strong correlation among generation means between the two forms of aggression, suggesting that the same loci affect both (genetic correlation: 0.98). In contrast, juvenile and territorial aggression in males were not strongly correlated among generation means of the JH, JL, TH, and TL lines (Fig. 12.4(b)), suggesting that in males, juvenile aggressiveness is only partly governed by the same genetic factors as is territorial aggression (genetic correlation: 0.50). The correlation between territorial and courtship aggression among generation means of the TH and TL lines also points to common genetic influences between these two forms of aggression (Fig. 12.4 (c); Sevenster 1961).

Selection for dominance ability produced little correlated change in other forms of aggressiveness; DH and DL line fish did not differ significantly in any of the other forms of aggressiveness (Bakker 1986). This result suggests that dominance ability is affected by other genetic factors than are juvenile, territorial, or courtship aggressiveness. The zero genetic correlation between juvenile aggressiveness and dominance ability was further substantiated by the outcomes of interline dominance tests in the third generation of selection. JH and JL males had similar dominance abilities to those of DH males (y-axes of Fig. 12.5(a),(b); Bakker 1986). Bidirectional selection for territorial aggressiveness resulted, however, in parallel changes in dominance ability; TH males had dominance abilities that were similar to (or even higher than) the dominance abilities of DH
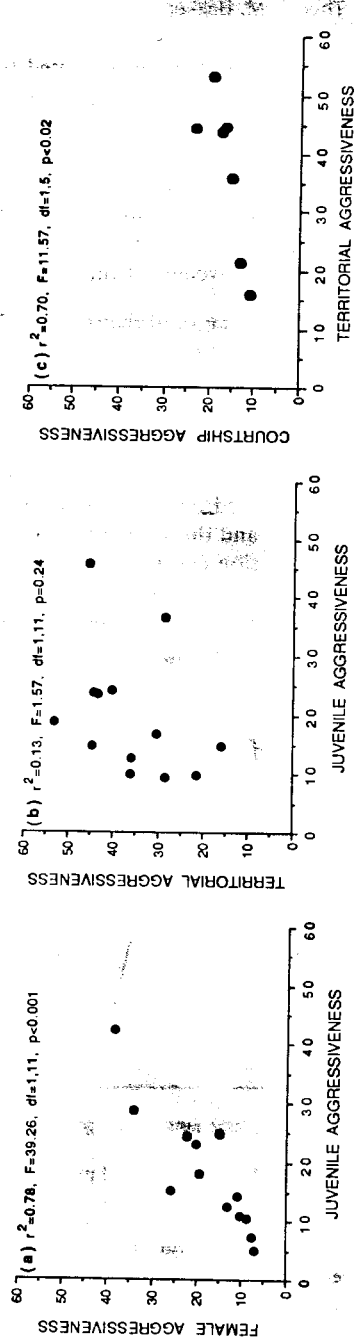
Fig. 12.4 Correlations between (a) juvenile and female aggressiveness, (b) juvenile and territorial aggressiveness, and (c) territorial and courtship aggressiveness. The data points on each graph represent the means of the aggression scores of all individuals in a single generation in a selected line. Three generations are represented for each of four selected lines: those selected respectively for high and low juvenile aggression (graphs (a) and (b)) and those selected respectively for high and low territorial aggression (graphs (a), (b), and (c)). A single mean was calculated for the control line using scores of individuals in the base population and in the second generation of the control line. See the text for additional details.

males, while the dominance abilities of TL males were similar to those of DL males (y-axes of Fig. 12.5(a),(b); Bakker 1986). This outcome seems to contradict a zero genetic correlation between territorial aggressiveness and dominance ability (see above). In this population, dominance ability correlates positively with the degree of red breeding coloration (Bakker and Sevenster 1983; Bakker 1986; see below). Also in the interline comparisons of dominance ability, differences between lines in the degree of red coloration explain the greater part of variation in dominance ability (Fig. 12.5(a),(b)). These results suggest a different genetic causation of colour changes in the territorial aggression lines and the dominance lines, leading to a zero genetic correlation between territorial aggressiveness and dominance ability in the dominance lines, but a positive one in the territorial aggression lines.

In combination, these studies demonstrate the existence of substantial genetic variation in aggressiveness of threespine stickleback. Natural and sexual selection acting on this variation has the potential to change the levels of aggression within and between populations. Indeed, parallel differences in the aggressiveness of juveniles from two anadromous and two freshwater populations suggest that natural selection may have done so. The genetic correlations among different forms of aggression are comparable in sign and magnitude with the corresponding phenotypic correlations (Bakker 1985, 1986), as usually is the case (e.g. Falconer 1981; Cheverud 1988). Stickleback aggression is thus characterized by a complex genetic correlation structure among different forms of aggression which may constrain the evolutionary response of specific forms of aggression. Evolution of stickleback aggression can best be understood by taking this complexity into account. Analyses of hormonal influences on stickleback aggression (see below) make clear that the genetic correlation structure of aggression is part of a larger complex involving reproductive behaviours and several life history characters.

Hormonal influences on aggression

The hormonal influences of the pituitary–gonadal axis on aggression by male threespine stickleback have been studied extensively. Gonadotropins are pituitary hormones, whose secretion is triggered by light (e.g. Slijkhuis 1978; Borg et al. 1987c). 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). This also holds when male stickleback that have been maintained under short photoperiods are stimulated into breeding condition by long photoperiods. It is only during the breeding season (long photoperiod, relatively high temperatures) that androgens inhibit gonadotropic cells (Borg et al. 1985; Guderley page 105 this volume).
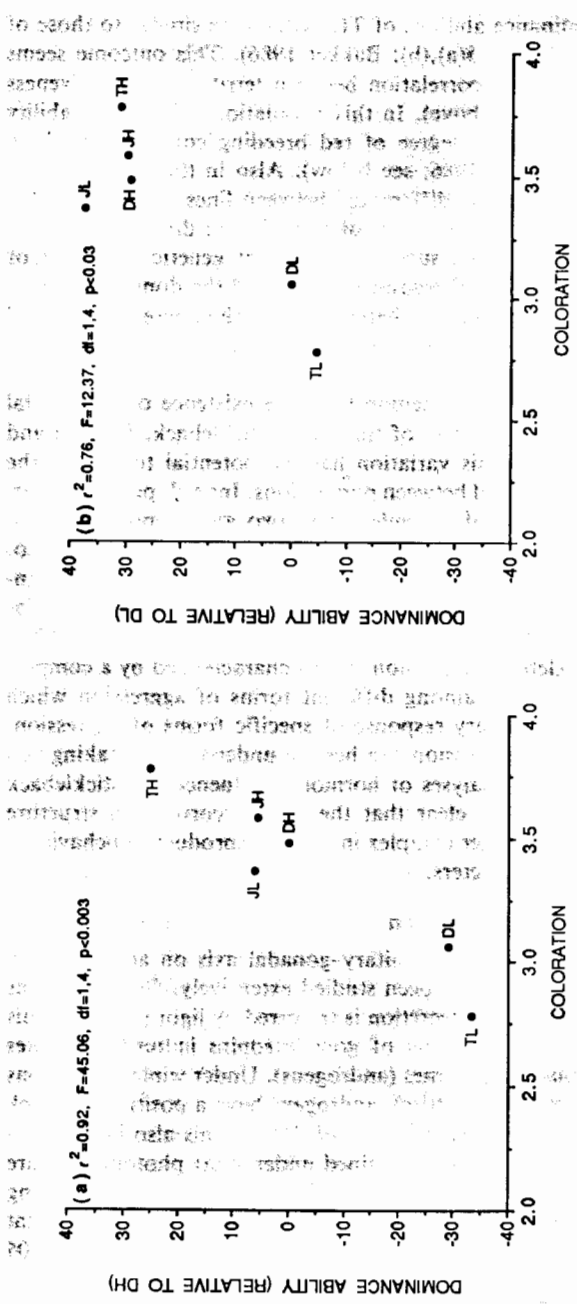
Fig. 12.5 Correlations between degree of red breeding coloration and dominance ability. The data points represent the means of 12–24 tests in the third generation of selection in a selected line. Dominance ability of selected lines is expressed as deviations from random (50 per cent of tests won by males of a selected line) in dominance tests against males of (a) the high dominance line (DH) and (b) the low dominance line (DL). The degree of red coloration of both contestants was scored on a four-point scale after the test. Scoring was conducted in the males' home tanks after presentation of a ripe female in a tube. JH, JL: high and low juvenile aggression lines, respectively; TH, TL: high and low territorial aggression lines, respectively.

In pre-breeding males, the level of aggression is related to the level of gonadotropins rather than to that of androgens (Hoar 1962a,b; Wai and Hoar 1963; Baggerman 1966, 1968; Wootton 1970). A combination of several methods (manipulation of photoperiod, castration of males, administration of testosterone or gonadotropins) was used to show this. For example, castrated, pre-breeding males (long photoperiod) are as aggressive as their intact counterparts, indicating that androgens are not important determinants of male aggressiveness at this stage.

Hormonal control of territorial aggression: controversial data?

The hormonal control of territorial aggression in the sexual phase (after nest building but before the start of the parental cycle) seems less clear. Males that are castrated or treated with anti-androgens in this phase show a drop in aggressiveness (Fig. 12.6; Baggerman 1966, 1968; Wootton 1970; Rouse *et al.* 1977), suggesting that androgens influence territorial aggressiveness. The variances within the sham-operated and castrated groups were high in Wootton's experiments, however, and the difference was not significant (Wootton 1970). In additional castration experiments, Wootton (1970) did document a decline in bites directed at stimulus males by empty-nest males measured at weekly intervals following gonadectomy. Unfortunately, no statistics are provided, but the error bars (Fig. 3 in Wootton 1970) suggest
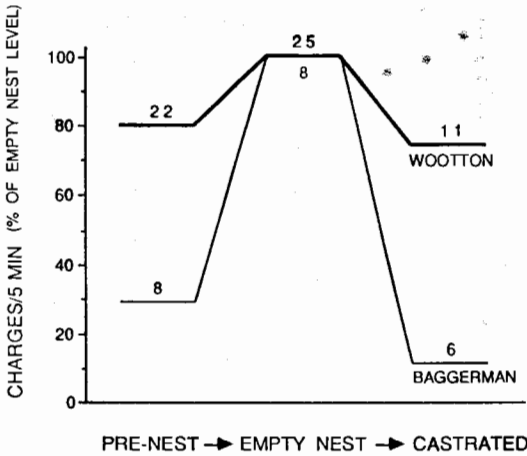
Fig. 12.6   Mean levels of aggression (charges per 5 min) of pre-nest, empty-nest, and castrated (empty-nest) males relative to those of empty-nest males in studies by Baggerman (1966) and Wootton (1970). The numbers of males tested are indicated in the figure. Data were estimated from Wootton, Table I and Fig. 2, and from Baggerman, Fig. 2, 5, and 7 (pre-nest: average of four tests during the 12 d period before nest building; empty nest: average of seven tests in the first 21 d after nest building; castrated: average of seven tests during the 21 d period after castration).

a significant effect of castration on aggressiveness. Baggerman (1966, 1968) did not test the significance of the decline in her experiments, but as her castrations had a greater effect than did those in Wootton's study (Fig. 12.6), the decline is probably significant in spite of the small sample size. Thus, the castration experiments suggest that androgens play a role in regulating aggressiveness at the empty nest stage. It is clear that additional experiments are needed to further test this hypothesis. Gonadectomy can be accomplished with minimal injury because the stickleback male's testes are more accessible than in most other fish species; this makes the threespine stickleback ideal for behaviour–endocrinological research (Borg pers. comm.).

An alternative interpretation of the decrease in aggressiveness following castration is that it could have been caused by loss of the nest rather than changes in androgen levels (Wootton 1970). Removal of a male's nest in the empty nest stage can cause aggression to decline (Symons 1965; Stanley and Wootton 1986), and castrated males do not maintain their nests. Although the nest may be a causal factor in determining the levels of aggression in nesting males, other findings counter the interpretation that the nest is the only causal factor involved. The establishment of dominance relationships between reproductive males in tanks unfamiliar to the contestants can be accompanied by intense fights (Bakker and Sevenster 1983; Bakker 1986). Additionally, parallel changes in kidney size in threespine stickleback males selected for high or low levels of territorial aggression suggest that androgens play a role as well (Bakker 1985, 1986; see below).

The effect of castration was much more pronounced in Baggerman's (1966, 1968) than in Wootton's (1970) study, leading to different interpretations as to the role of gonadotropins in controlling aggressiveness of empty-nest males. In Baggerman's study, the level of aggression of empty-nest males following castration was reduced to very low levels (Fig. 12.6). Her interpretation of this was that there had been a switch in the hormonal control of aggressiveness, such that androgens took over the role of gonadotropins and directly affected aggression during the sexual phase. Wootton (1970) pointed out a problem with this interpretation. He noted that the level of aggression of the castrated males did not drop below that of pre-nesting males (Fig. 12.6), suggesting that the same causal factors that determined pre-breeding aggressiveness were still in effect. Baggerman's data also fit in with this interpretation, because it is unlikely that in her study the levels of pre-breeding aggression and aggression after castration differed significantly.

The disagreement as to whether gonadotropins influence aggressiveness during the sexual phase arose because levels of pre-breeding aggression differed between studies. Apart from some other methodological differences, Baggerman also used fish from a Dutch anadromous population whereas Wootton's fish came from a British freshwater population. It has

been suggested several times that population differences caused the discrepancy between the studies (Liley 1969; Munro and Pitcher 1983; Villars 1983). Indeed, parallel differences in the levels of aggression before breeding have been reported for anadromous and freshwater populations from the Netherlands and Japan (see above). It will be argued in the next section that in the case of the Dutch populations, the differences are due to different levels of gonadotropins.

The most plausible overall picture that emerges is that a stickleback male's aggression is controlled by gonadotropins outside the reproductive period, and by both gonadotropins and androgens during the reproductive period. For a proper evaluation of the evolution of aggressiveness in threespine stickleback, it is crucial that we understand the causal mechanisms leading to variation in aggressiveness. The pituitary–gonadal axis plays a key role in this, not only by controlling aggressiveness in different phases in the life cycle, but also by controlling sexual behaviour, the physiological capability for reproduction, and the male's secondary sexual characters (Guderley page 108 this volume). The evolution of aggressiveness may therefore be influenced indirectly through the pituitary–gonadal axis as a consequence of selection on different aspects of phenotype.

The endocrine basis of genetic correlations: constraints on the evolution of aggression?

Differences in life history and morphological traits among the lines selected for different forms of aggression provided evidence of the involvement of two classes of hormones in control of aggression (Bakker 1985, 1986). Juvenile aggressiveness was negatively correlated with the age at sexual maturity among generation means of the low and high juvenile aggression lines (Fig. 12.7; Bakker 1986). Thus, selection for juvenile aggressiveness was accompanied by a change in the age at sexual maturity, such that JH fish matured earlier than JL fish (age at sexual maturity in the third selected generation in days after fertilization: JH males, $\bar{X} \pm SD = 117.8 \pm 13.8$, $N = 15$; JL males, 130.7 ± 17.3, $N = 11$; JH females, 121.9 ± 15.8, $N = 17$; JL females, 134.6 ± 19.3, $N = 12$; Mann–Whitney U test, one tailed, $U = 46$ and 61, respectively, $P < 0.04$ for both sexes) (Bakker 1986). Additionally, the onset of juvenile aggression (in d after fertilization) was later in JL fish, as assessed in the third selected generation in standardized groups of juveniles (JH, $\bar{X} \pm SD = 34.7 \pm 1.9$, $N = 6$; JL, 41.8 ± 3.4, $N = 9$; Mann–Whitney U test, two tailed, $U = 0$, $P < 0.002$) (Bakker 1986). Finally, the incidence of female ripeness was lower in JL fish than it was in JH fish (Fig. 12.8). 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 vitello-
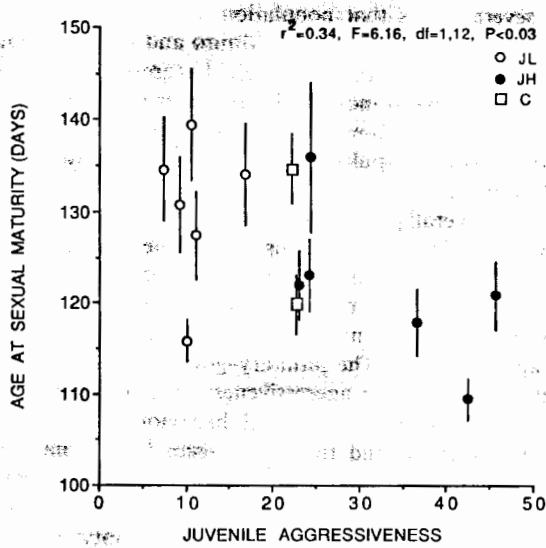
Fig. 12.7 Correlation between juvenile aggressiveness and age at sexual maturity (in days after fertilization). The data points represent the means of all males or females in successive generations of the juvenile high (JH) and low (JL) aggression lines and the second generation of the control (C) line. Sexual maturity was defined for males as completion of the first nest and for females as spawning readiness for the first time. Error bars represent two standard errors of the mean.

genesis, ovarian oestrogen secretion, oocyte maturation, and ovulation in females (reviewed in e.g. Idler and Ng 1983; Ng and Idler 1983). Some of these functions of gonadotropins are impressively demonstrated in the platyfish, *Xiphophorus maculatus*, in which several alleles at a single locus affect the development of the gonadotropic cells in the pituitary gland (e.g. Schreibman and Margolis-Nunno 1987; Schreibman *et al.* 1989). Various combinations of alleles at this locus determine, for instance, the age at which sexual maturity occurs (between 8 and 104 wk).

Attainment of sexual maturity was delayed in the JL 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 or stops after attainment of sexual maturity (e.g. Wootton 1976, 1984*a*, Crivelli and Britton 1987; Mori and Nagoshi 1987); later-maturing individuals tend to be larger.

Finally, females in the JH line appeared to mature clutches more rapidly than did females in the JL line. This could indicate a genetic correlation
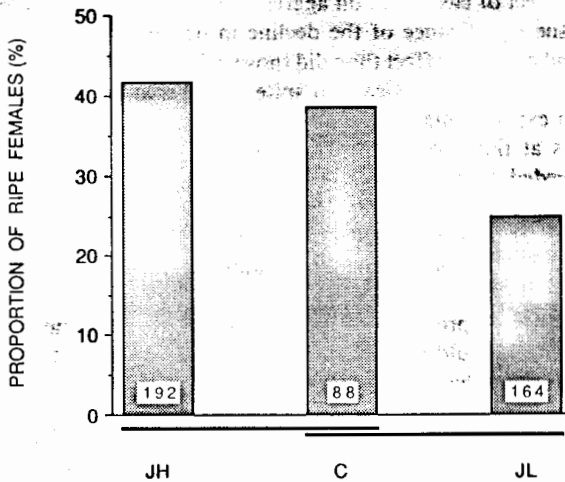
Fig. 12.8 The proportions of ripe females in the high (JH) and low (JL) juvenile aggression lines and in the control (C) line. The proportions were based on the number of ripe females in four female aggression tests (one per week) after the females became ripe for the first time. Data from females in all three generations were pooled within each selection line. The numbers of aggression tests are indicated at the bases of the histogram bars. The overall data set was heterogeneous ($G = 11.7712$, d.f. $= 2$, $P < 0.005$). Lines below the x-axis connect treatments that did not differ significantly at the 0.05 level (STP procedure of Sokal and Rohlf 1981; $G_H < 5.701$, d.f. $= 1$, $k = 3$).

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 1973b). Data from the JH, JL, and C lines in the second selected generation (Bakker, unpubl. data), where differences among lines in the level of juvenile aggression were greatest (Fig. 12.2), give some support to the former explanation. Females selected to mother the next generation matured later and tended to be larger in the JL line than in the JH line (age at sexual maturity ± SD: JL, 157.0 ± 19.1 d after fertilization, $N = 3$; JH, 108.3 ± 9.7, $N = 3$; Mann–Whitney U test, one tailed, $U = 0$, $P = 0.05$; length ± SD: JL, 6.1 ± 0.3 cm; JH, 5.6 ± 0.4; Mann–Whitney U test, one tailed, $U = 1.5$, $P = 0.15$) or C line (age at sexual maturity 131.8 ± 17.4 d, $N = 9$, Mann–Whitney U test, one tailed, $U = 3.5$, $P < 0.05$; length 5.8 ± 0.2 cm, Mann–Whitney U test, one tailed $U = 5$, $P = 0.097$). Although there was some variation in age at which lengths were assessed (JL, 288.7 ± 4.6 d after fertilization; JH, 271.3 ± 18.5; C, 307.9 ± 12.7), this does not account for the observed differences. One

to two months before the length assessments (age: JL, 241.0 ± 10.1 d after fertilization; JH, 217.8 ± 28.1; C, 246.7 ± 21.1), JL females also produced larger clutches than did JH females (JL, $\bar{X} \pm SD = 180.0 \pm 26.6$ eggs; JH, 143.5 ± 5.1; Mann-Whitney U test, one tailed, $U = 0$, $P = 0.05$) or C females (132.4 ± 27.1 eggs, Mann-Whitney U test, one tailed, $U = 2$, $P = 0.025$). The smaller clutches produced by JH females seemed to be more than compensated for by a higher clutch maturation rate (Fig. 12.8). In the second selected generation, the difference between per cent ripe JH and JL females during the tests was even greater (Bakker 1986). Thus JL females enjoyed a greater clutch size, but lifetime fecundity was probably greater in JH females.

Selection on territorial aggressiveness seems to have affected the level of androgen production rather than the level of gonadotropin production. TH line males have enlarged kidneys relative to control line males, a condition indicative of elevated levels of androgen production (Wai and Hoar 1963; Moufier 1972; de Ruiter and Mein 1982), whereas TL males have smaller kidneys than those of the controls (Fig. 12.9). Parallel changes in the degree of red breeding coloration in TH and TL males (Fig. 12.5) further support this hypothesis.
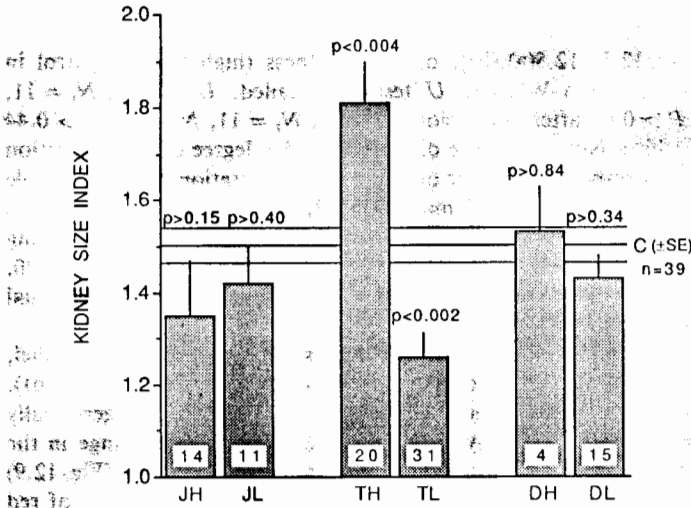

Fig. 12.9 Mean kidney size index of males in the fourth selected generation of the juvenile high (JH) and low (JL) aggression lines, territorial high (TH) and low (TL) aggression lines, and in the high (DH) and low (DL) dominance lines. The numbers of males examined are indicated at the bases of the histogram bars. Error bars indicate one standard error of the mean. Relative kidney sizes in the selected lines were compared with that of the control (C) line by the Mann-Whitney U test, two tailed.

Although genetic correlations between aggressiveness and most aspects of reproductive behaviour have yet to be investigated, their common hormonal control and signalling system (red nuptial coloration of the male) make it likely that some aspects of aggressive and reproductive behaviour will prove to have evolved in concert. The probable hormonal causes of such character correlations are the androgens, which have been implicated as determinants of aggressiveness, nest building, nest-directed activities, courtship behaviour, and the secondary sexual characteristics of the male (Craig-Bennett 1931; Ikeda 1933; Hoar 1962a,b; Wai and Hoar 1963; Baggerman 1966, 1968; Wootton 1970; Rouse et al. 1977; Borg 1981, 1982a, 1987; Borg et al. 1987c, 1989c; Andersson et al. 1988; Mayer et al. 1990a; Guderley page 105 this volume). Thus, androgen levels, levels of some forms of aggression, nuptial coloration, and reproductive behaviour tend to covary over the life cycle of the male. It also follows that differences in androgen levels among individuals or populations can also lead to positive correlations between these characters (Rowland 1984; Giles and Huntingford 1985; McLennan and McPhail 1989b).

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 above). For this reason, not all forms of aggression are positively correlated with reproductive behaviour. For example, in lines selected for low levels of juvenile aggression (JL) and low levels of territorial aggression (TL), males of the third and fourth selected generations displayed similarly low levels of aggressive activity during courtship. However, JL and TL males differed significantly in direct (courtship intensity expressed as the number of zigzags) and indirect (particular nest-directed activities) measures of sexual tendency; compared with males of the control line or of the corresponding high line, JL males tended to display an enhanced sexual activity, whereas the sexual activity of TL males tended to be reduced (Bakker and Sevenster 1989).

These findings support the interpretation that levels of juvenile aggression are affected primarily by gonadotropin levels, whereas territorial aggression is also affected by androgen levels. 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 above). Because 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 stickleback (Sevenster 1968, 1973; Peeke 1969; van den Assem and van der Molen 1969; Wilz 1972; Rowland 1988; Bakker and Sevenster 1989), a one-sided reduction of the aggression level as in JL males 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.

A comparison of the Dutch freshwater and anadromous populations (see above) gives some insight into the evolutionary implications of the different involvement of gonadotropins and androgens in the aggressive and sexual behavioural systems. The laboratory-bred freshwater fish were more aggressive and territorial during the juvenile stage (Fig. 12.1), and freshwater males had higher levels of territorial aggression but lower levels of courtship activity (see above). The differences in levels of territorial aggression did not appear to be attributable to differences in androgen levels, as mean kidney size in the anadromous population (kidney size index $\pm$ SD = 2.06 $\pm$ 0.32; N = 25) did not differ significantly from that in the freshwater population ($\bar{X}$ = 2.07 $\pm$ 0.22; N = 37; Mann–Whitney U test, one tailed, U = 455, Z = 0.108, P > 0.45). Because laboratory-reared individuals from the two populations differed markedly in their levels of aggression during the juvenile stage (Fig. 12.1), it is likely that differences in the level of (or sensitivity to) gonadotropins were responsible for the difference in territorial aggressiveness between the populations. The higher sexual activity of the anadromous males agrees with this interpretation. These results suggest that selection for reduced or enhanced aggression in juvenile stickleback has implications for aggression and sexual activity of mature fish.

Aggressive and reproductive behaviour also appear to be linked by the dual signalling role of the male's red breeding coloration in at least some populations. Early research in which dummies were presented inside the territories of males demonstrated an aggression-releasing effect of the red undersides of male threespine stickleback (ter Pelkwijk and Tinbergen 1937; Tinbergen 1948, 1951; see also Baerends 1985; Collias 1990), causing Tinbergen to conclude that this coloration was the primary sign stimulus for territorial aggression in this species. This interpretation has been questioned, however, because several recent studies failed to detect an aggression-releasing effect of the red belly (Rowland page 300 this volume).

One explanation for the differences among studies may lie in the intimidating effect of the male's red coloration. Tinbergen (1948) found that a dummy with a red belly was more effective in inhibiting rival males from entering the territory than was a non-red dummy. More recent research has suggested that dominance ability of males may also be correlated with the development of the red coloration (Bakker and Sevenster 1983; Bakker 1985, 1986), but again, not all studies have documented such a relationship (FitzGerald and Kedney 1987; Rowland 1989a).

Intriguingly, the successful selection for low dominance ability in male stickleback (Fig. 12.3) was paralleled by a significant reduction in the degree of red breeding coloration (Fig. 12.10(a),(b); Bakker 1986) (high versus low in generation 3, Mann–Whitney U test, one tailed, U = 43.5, N_1 = 14, N_2 = 13, P < 0.01 after aggression; U = 56, N_1 = 13, N_2 = 15, P < 0.03 after courtship). The DH line showed no increase in dominance
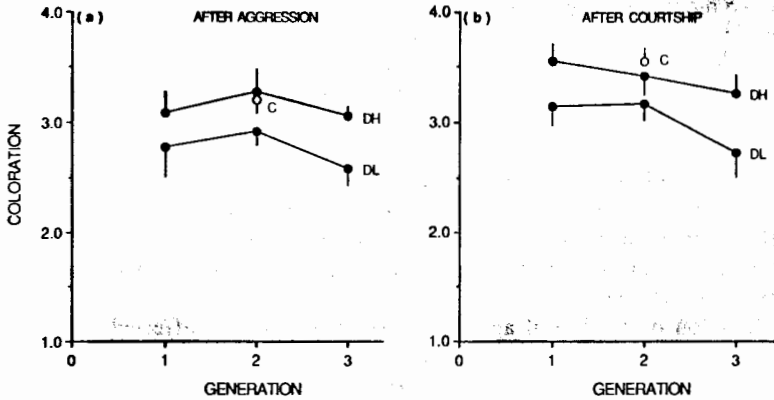
Fig. 12.10 The mean degree of red breeding coloration of males in three successive generations selected for low (DL line) and high (DH line) levels of dominance ability, and in the second generation of the control (C) line. Degree of red coloration was scored (a) after presentation of a male in a tube and (b) after presentation of a ripe female in a tube. Error bars indicate one standard error of the mean.

ability (Figs. 12.3, 12.5(a),(b)), or in brightness (high versus control in generation 2, Mann–Whitney U test, two tailed, $U = 78.5$, $N_1 = 11$, $N_2 = 16$, $P > 0.63$ after aggression; $U = 73$, $N_1 = 11$, $N_2 = 16$, $P > 0.44$ after courtship). Notice also the difference in the degree of red coloration of all males, irrespective of their origin, after presentation of a ripe female (Fig. 12.10(b)) and of a rival male (Fig. 12.10(a)); females make males flush more strongly (Wilcoxon matched-pairs signed-ranks test involving generations of the DH, DL, and C lines, $T = 629$, $N = 83$, $Z = 5.070$, $P < 0.001$). This difference points to the role of red coloration in intersexual selection (see below).

Bidirectional selection on territorial aggression also produced parallel, bidirectional differences in degree of red coloration (Fig. 12.5(a),(b)), suggesting that this character can be modified through two genetically distinct physiological pathways (Bakker 1986). The colour change in the low dominance line is likely due to androgen-independent factors (Fig. 12.9) that, for example, influence the concentration and/or distribution of red and other pigments, rather than to the probably androgen-dependent factors that influence coloration in the territorial aggression lines.

Some of the discrepancies among studies on the aggression-releasing effect of red coloration are probably due to methodological differences, because the red coloration on a male or on a dummy can either elicit aggression or inhibit attack. Differences in the quality of the red colour or in presentation could alter the response of territorial males (Rowland page 300 this

volume). Alternatively, the discrepancies could reflect population differences. The only study that compared the response of males from different populations towards dummies with and without a red belly did find, however, a similar intimidating effect of a red belly in males from different populations, i.e. in males from a marine (brackish) population sampled on Long Island, New York, USA, and in those from a mixture of Dutch freshwater and anadromous males (Rowland and Sevenster 1985).

In contrast, there is evidence of a difference in the intimidating effect of red coloration between a Dutch freshwater and a Dutch anadromous population. Laboratory-reared anadromous males tended to be slightly, but significantly brighter than laboratory-reared freshwater males, suggesting a genetic basis to the difference. Their dominance ability was, however, less than that of their freshwater counterparts (Table 12.1). When linear orders of dominance were constructed for the males in each population, there existed a significant positive correlation between the degree of red coloration and dominance ability in the freshwater population (Fig. 12.11(a)) but not in the anadromous population (Fig. 12.11(b)). The former correlation was not attributable to particular males (jackknifing correlation coefficients ranged from 0.61, $N = 9$, $P < 0.05$, to 0.85, $N = 9$, $P < 0.01$). Body size and aggressiveness were both uncorrelated with dominance ability in these populations (Bakker and Feuth-de Bruijn unpubl. data).
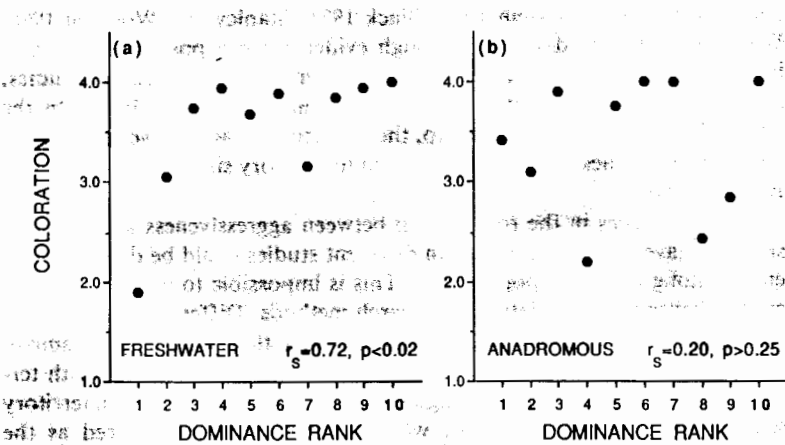
Fig. 12.11 The relationship between dominance rank and degree of red breeding coloration among ten males from (a) a Dutch freshwater population and (b) a Dutch anadromous population. Dominance rank was assessed in dyadic combats between isolated males, and mean degree of red coloration was quantified on a four-point scale after each dominance test upon stimulation with an enclosed ripe female. r_s is the Spearman rank correlation coefficient.

The male's red coloration has also been shown to function in intersexual selection in threespine stickleback (see also Rowland page 317 this volume). Ter Pelkwijk and Tinbergen (1937) noticed that there was little response of ripe females to male dummies that lacked a red belly. Subsequently, Semler (1971) demonstrated that females from Wapato Lake, Washington, USA, a population in which males were polymorphic for the development of red nuptial coloration, preferred red over non-red males. It was only recently shown that female stickleback prefer the more intensely red-coloured male in simultaneous (McLennan and McPhail 1990; Milinski and Bakker 1990) and sequential choice situations (Bakker and Milinski 1991). Using fish from a Swiss freshwater population (near Roche/Montreux; probably anadromous fish introduced at the end of the 19th century, Bakker unpubl. data) in choice tests under two light conditions, Milinski and Bakker (1990) showed that female mating preference is based almost exclusively on the intensity of the male's coloration, which reveals his physical condition.

Clearly then, red male nuptial coloration functions as a signal both in aggressive interactions with conspecifics and in mate choice. Although it has been suggested that the evolution of colour patterns in the Gasterosteidae is more strongly correlated with intersexual selection than with intrasexual selection (McLennan *et al.* 1988), the apparent signal function of the red nuptial coloration in aggressive interactions among males suggests that in threespine stickleback, intrasexual selection could also have affected the evolution of this trait. This conclusion obviously applies only to the intimidating effect of red, because it is very unlikely that its aggression-releasing effect has played a role in the evolution of red breeding coloration in threespine stickleback; it may be rather disadvantageous to its bearer to provoke aggression of rivals. Equally clearly, because red nuptial coloration serves a signal function in both intrasexual aggression and courtship, selection on either could produce a correlated change in the other. As a result, it may prove difficult to discriminate the relative importance of selection on the two signal functions of this character.

NATURAL SELECTION, SEXUAL SELECTION, AND THE EVOLUTION OF AGGRESSIVENESS

Among the sticklebacks, *G. aculeatus* has the best developed morphological defence mechanism against vertebrate predators (e.g. Hoogland *et al.* 1957; Nelson 1971; Bowne Chapter 2 this volume) and at the same time the highest levels of aggression and the most pronounced breeding coloration (e.g. McLennan *et al.* 1988). There exists a consensus that predation is a major selective force in both morphological and behavioural evolution of the threespine stickleback (Reimchen Chapter 9; Huntingford *et al*, Chapter 10 this volume). Because of the well-developed morphological and

behavioural defences observed in many populations, it has been argued that the intensity of predation may be lower in this species than in other species of sticklebacks (but see Reimchen page 254 this volume, for evidence of heavy predation loads in some populations). On this basis it has been suggested that the relative freedom from predation might have facilitated the emancipation of reproductive males from nesting in areas of dense vegetation to the open (Morris 1958; Wilz 1971; Wootton 1976, 1984a) where competition for females would be more intense.

If correct, this scenario suggests that relative freedom from predators, in some populations at least, could have permitted the evolution of male traits that enhance their competitive abilities (e.g. high aggression levels) and attractiveness towards females (e.g. red breeding coloration). As these characteristics impose temporal and energetic costs and increased predation risk, there must be a net advantage to nesting in breeding aggregations in open habitats. Under laboratory conditions, males with near neighbours develop more intense red breeding coloration (Reisman 1968b, Sargent 1985), and are more motivated to court females late in the brood cycle (Sargent 1985) than are solitary males. Furthermore, males in nesting aggregations in nature may be able to attract more females (Kynard 1978a; Mori 1990b) and to detect sneakers at a greater distance (Mori 1990b) than can solitary males. These factors could result in higher lifetime reproductive success for gregarious males (Sargent 1985; Sargent and Gross 1986).

The costs of aggression

There can be little question that aggressiveness should be subject to natural selection, at least in part because aggression is energetically costly. Chellappa and Huntingford (1989) demonstrated that participation in a fight of more than a few seconds' duration left both males with depleted liver glycogen. In short fights, the level of liver glycogen was lowest in the defeated male. As the fights increased in length, the liver glycogen levels decreased, as did the difference in levels between the males. Similarly, under laboratory conditions, males that held territories in isolation lost weight less rapidly during the brood cycle than did males with neighbours (males were separated by a transparant partition), suggesting a cost of territorial aggression (Sargent 1985; Sargent and Gross 1986). Males that were fed more expanded the territories they defended (Stanley and Wootton 1986).

So far as involvement in aggressive interactions affects physical condition, it may affect the intensity of breeding coloration (Milinski and Bakker 1990) and hence courtship success (see above). The fitness of reproductive males should be enhanced by minimizing the energetic costs they incur during aggressive interactions. The frequency and intensity of aggressive interactions could be reduced through habituation of aggression against familiar rivals (neighbours), allowing males to maintain intense red breeding coloration and high levels of sexual activity (see below; Rowland 1988).

Habituation is probably only possible after neighbouring males have assessed the fighting abilities of one another when these abilities are signalled reliably (e.g. through the intensity of breeding coloration, which is positively correlated with physical condition; Milinski and Bakker 1990) and when aggressive intentions are signalled honestly (Losey and Sevenster 1991).

For other reasons as well, high levels of aggression are not always advantageous. Under laboratory conditions, when territories are being established, highly aggressive males are not more likely to obtain territories than are less aggressive males (Muckensturm 1969; Bakker and Sevenster 1983; Bakker 1986; FitzGerald and Kedney 1987; Rowland 1989a). Red breeding coloration (Bakker and Sevenster 1983; Bakker 1986) or large body size (Rowland 1989a) may be more important determinants of the outcome of competition for territories.

Furthermore, costs of aggression probably include increased risk of predation owing to reduced vigilance and increased conspicuousness, and interference with courtship activity (see below).

Territorial aggression and determinants of reproductive success

Territorial aggression and territory size

High aggression levels at relatively great distances from the nest do appear to be necessary for maintenance of a large territory under laboratory conditions (van den Assem 1967; Black 1971; Stanley and Wootton 1986; Ward and FitzGerald 1988), although evidence for a positive association between territory size and aggressiveness is mixed. In laboratory studies, the relationship was positive (van den Assem 1967; Black 1971). In the only field study of this relationship, the frequency of aggressive interactions between rival males was inversely related to territory size (Goldschmidt and Bakker 1990).

The differences in the relationship between aggressiveness and territory size that have been documented in different studies could be due to differences among the study populations. This is impossible to determine, however, because of the variation in research methods. Differences in methods of measuring aggression can substantially alter the results. For example, Black (1971) found that aggressiveness was positively associated with territory size, and that changes in aggressiveness paralleled changes in territory size during the parental phase, when aggressiveness was scored as the number of aggressive interactions between males sharing an aquarium. In contrast, tests in which a rival male was presented in a tube 20 cm from the nest and aggression was measured as the number of bites and bumps per 5 min revealed no correlation between territory size and aggressiveness (Black 1971).

Additionally, the measure of aggressiveness differed among studies, as did the accuracy with which these different measures were likely to reflect

male aggressiveness. For example, counts of the number of aggressive interactions between males and freely moving conspecifics are likely to be affected by immediate environmental conditions, such as the amount of cover near a nest or the number of foraging individuals moving through the area at the moment. A final problem with comparison of these studies is that the relationships between aggression and territory size were scored at different stages in the reproductive cycle. A further complication is that a large body size and intense red breeding coloration probably contribute to the maintenance of a large territory by signalling superior fighting ability, in the case of coloration through its positive correlation with physical condition (Milinski and Bakker 1990; also implicitly suggested by studies of Rowland 1984; McLennan and McPhail 1989*b*). The relationship between male aggressiveness and territory size under natural conditions could best be resolved with controlled field experiments.

Reproductive tactics

In homogeneous habitats, superior reproductive success is associated with large territories, both in the laboratory (van den Assem 1967; Black 1971; Li and Owings 1978*a*) and in the field (Goldschmidt and Bakker 1990). Males with large territories experienced greater mating success (van den Assem 1967; Li and Owings 1978*a*) and reared more progeny (van den Assem 1967; Black 1971) in the laboratory than did males with smaller territories. Similarly, males with large territories in a trout pond in the Netherlands experienced greater mating success and suffered less egg raiding than did those with smaller territories (Goldschmidt and Bakker 1990).

The advantages of large territories include lower rates of aggressive interactions initiated by neighbours and lower rates of sneaking by rival males (van den Assem 1967; Goldschmidt and Bakker 1990). These interactions decrease reproductive success directly by interrupting courtship and through loss of fertilizations to sneaking males that enter the nest and release sperm over the eggs. In addition, these interactions stimulate aggressive behaviour in the owner of the territory, thereby slowing courtship (van den Assem 1967; Black 1971; Li and Owings 1978*a,b*; Ridgway and McPhail 1987). As there exists a mutually inhibitory relationship between the tendencies to show aggressive and sexual behaviour (see above), a high aggression level decreases a male's willingness to lead a ripe female to his nest. Recent aggressive interaction causes a prolongation of courtship and an increased risk that the female will depart before spawning (e.g. van den Assem 1967; Wilz 1970*a,b*, 1972; Borg 1985; Ward and FitzGerald 1987).

This negative relationship between aggressive and reproductive behaviour was also evident in tests of female mate choice when a small territory size was imposed by the design of the experiment. Aggressive males experienced lower spawning success than did those that were less aggressive, because females departed before spawning (Ward and FitzGerald 1987). It is clear

not only that aggression is energetically costly, but that during the courtship phase of the reproductive cycle, high levels of aggressiveness are also costly in terms of lost mating opportunities. As a result, selection against high levels of courtship aggression could produce decreases in other correlated aspects of aggression, holding them at lower levels than would otherwise be favoured by selection.

In homogeneous habitats, selection thus favours large territory size because it assures relatively undisturbed courtship. In contrast, FitzGerald (1983) and FitzGerald and Whoriskey (1985) documented an inverse relationship between internest distance (an indirect measure of territory size) in the field and the number of eggs in nests in a salt-marsh population in Quebec, Canada, which they interpreted as evidence of an inverse relationship between territory size and reproductive success. The negative correlation was, however, established in a heterogeneous habitat where the nests of males with small territories were protected by the banks of the tidal pools. Several studies have shown that concealment of nests increases reproductive success (Moodie 1972b; Kynard 1978a; Sargent and Gebler 1980; Sargent 1982; Mori 1990b). Nest concealment can seemingly also reduce the frequency of aggressive interactions during courtship and paternal care (Sargent and Gebler 1980). Nest concealment also decreases the frequency of courtship interference and the probability of stolen fertilizations and egg raids by sneakers (Sargent and Gebler 1980). Under such circumstances, selection for high levels of territorial aggression may be reduced.

The above review of the relationship between territorial aggressiveness and reproductive success opens the possibility of the existence of two reproductive tactics: a 'nest concealment' tactic and a 'large territory size' tactic, both leading to superior reproductive success by way of reduced interference from rivals. The nest concealment tactic would require neither such great dominance abilities (large body size and/or intense red breeding coloration) nor such high aggression levels as the large territory size tactic would. The nest concealment tactic would likely have a relative advantage in populations that are subject to a high vertebrate predation pressure (Huntingford et al. Chapter 10 this volume), whereas insect predators would likely reverse this advantage (Foster et al. 1988). Additionally, in populations where parasites exert a major influence, the large territory size tactic would possibly be favoured through intensified intersexual selection for bright red breeding coloration (Hamilton and Zuk 1982; Milinski and Bakker 1990). The presence of both strategies within stickleback populations could be one of the causes for a sustained genetic variation of aggressiveness.

Some support for the possible existence of two reproductive tactics comes from observations on a benthic–limnetic species pair of threespine sticklebacks in Enos Lake, British Columbia, Canada (McPhail page 421 this

volume). In this lake, territorial males of the limnetic species, which nests in open habitats, are frequently involved in aggressive interactions with conspecific neighbours. Such interactions are rare between benthic males that nest in vegetation. Consequently, courtship in the limnetic species is protracted relative to that in the benthic species because of a high frequency of interruptions by rival males (Ridgway and McPhail 1987).

In order to judge the impacts of different selection regimes on aggression, field studies that directly compare the fitness of different aggressive pheno-types in different contexts or in different populations are essential. No such studies have been conducted yet, although the threespine stickleback should be an excellent subject for such research. This small fish can readily be observed *in situ* in many aquatic habitats (e.g. Kynard 1978*a*; FitzGerald 1983; Borg 1985; Foster 1990), and different populations are exposed to differing selection regimes known to have produced divergence in other behavioural phenotypes (e.g. Huntingford *et al.* Chapter 10; Foster Chapter 13 this volume). The results discussed above on interpopulation differences in aggression suggest that at least some aspects of aggressive behaviour have also diverged among populations, but more research, involving field and laboratory observations on more populations, is needed.

Life history mode and the evolution of aggression

Differences in aggressiveness of individuals from two pairs of anadromous and freshwater populations suggest that there are differences in aggressive-ness among populations that reflect the selection regimes to which they are exposed (Honma and Tamura 1984; Bakker and Feuth-de Bruijn 1988). The lower levels of juvenile aggression measured in both anadromous populations relative to the freshwater populations could be favoured because juveniles in anadromous populations migrate to the sea in large shoals (e.g. Daniel 1985). High levels of intraspecific aggression might be disadvantageous in shoals, and this disadvantage could account for the seemingly lower aggression of anadromous stickleback (Bakker and Feuth-de Bruijn 1988), although freshwater juveniles are also known to participate in large feeding schools (e.g. Foster *et al.* 1988). Additional field and laboratory studies are necessary to determine the robustness of this seeming dichotomy between freshwater and anadromous threespine stickleback and to ascertain the causes.

In the Dutch comparison (Bakker and Feuth-de Bruijn 1988), juveniles in the freshwater population were also more likely to establish territories in the laboratory. Although territoriality of juveniles has not been docu-mented in the field, MacLean (1980) has observed defence of feeding territories in a Canadian lake outside the breeding season. Certainly, juvenile territoriality is more likely to be favoured in non-migratory than in migra-tory populations. Parallel differences in aggressiveness and territoriality have been observed in juvenile salmonids that migrate to sea immediately

after hatching and those that delay migration (Keenleyside 1979).

Anadromous and freshwater populations should exhibit differences in life history and behavioural characters, owing to the higher costs of a migratory lifestyle (e.g. Stearns 1976; Roff 1988). There is evidence for the existence of a so-called migratory life history syndrome, in which the relevant life history characters show positive genetic correlations (e.g. Dingle 1988). Several life history characters have been compared between anadromous and freshwater stickleback populations and they support the predictions (wild-caught fish: e.g. Hagen 1967; laboratory-bred fish: Snyder and Dingle 1989, 1990; Snyder 1990; Bakker and Feuth-de Bruijn unpubl. data). Lines selected for enhanced and reduced levels of juvenile aggression suggest that aggressiveness is genetically correlated with this migratory life history syndrome through gonadotropins (see above). The evolution of juvenile aggressiveness (and correlated forms of aggressiveness) may therefore be influenced indirectly through selection on life history traits, such as body size and age at reproduction.

Comparisons of marine (or anadromous) and freshwater populations have the potential to provide insight into the directions of evolutionary change, because freshwater populations are thought to have been derived from the marine form (e.g. McPhail and Lindsey 1970; Bell 1976a; Bell and Foster page 14; McPhail page 401 this volume). For example, all of the populations in which the intensity of red nuptial coloration proved not to be an important determinant of dominance ability among males were anadromous populations (FitzGerald and Kedney 1987; Rowland 1989a; Bakker and Feuth-de Bruijn unpubl. data). The only population that has been studied in which coloration played a prominent role in determining dominance relationships was a freshwater population (Bakker and Sevenster 1983; Bakker 1985, 1986; Bakker and Feuth-de Bruijn unpubl. data). It is therefore possible that in marine populations, nuptial coloration functions primarily to determine patterns of mate choice (intersexual selection), and that the signal function of this coloration in dominance contests is a derived condition. This possibility could be tested in a systematic study of the signal function of the male's coloration across a large number of marine and freshwater populations.

CONCLUSIONS

Although a limited number of studies have investigated genetic influences on variation in aggressiveness, there is clear evidence for genetically based variation in aggressiveness within and among threespine stickleback populations. Aggressiveness is part of a complex character suite in which different forms of aggressiveness, sexual behaviour, the intensity of red breeding coloration, and several life history characters are genetically correlated to varying degrees. The genetic correlations in this complex are partly based

on the multiple influences of hormones of the pituitary–gonadal axis. The evolution of aggressive behaviour can only be understood by taking this complex into account.

Different determinants of variation in reproductive success have been established, both in laboratory and in field studies. They suggest the possible existence of two reproductive tactics: a 'nest concealment' tactic and a 'large territory size' tactic, both leading to superior reproductive success by reducing interference from rivals. The tactics probably require different dominance abilities and territorial aggression levels, and their relative advantages are likely to depend on the presence and abundance of vertebrate and insect predators.

In addition, selection processes acting on aggressiveness in the juvenile stage and of adult females also have some impact on the evolution of aggressiveness in stickleback. The importance of aggression in juveniles and females leads to the notion that different life history modes, which are accompanied by differences in life history and behavioural characters (e.g. juvenile aggressiveness), may have hitherto unrecognized evolutionary consequences for stickleback aggression.

The proposed influences of different reproductive tactics and life history modes on the evolution of stickleback aggression generate testable hypotheses for future research.

ACKNOWLEDGEMENTS