Parasitism of asymmetrical pelvic phenotypes in stickleback

T.E. Reimchen

Abstract: Subtle departures from bilateral symmetry in morphological traits result from environmental and genetic stresses and may signal an inferior genetic background. Because one correlate of an inferior genome is reduced resistance to infection, such asymmetry may provide a phenotypic signal of susceptibility to parasitism. I tested this hypothesis in a population of threespine stickleback (*Gasterosteus aculeatus*) with cestode and nematode infections and bilateral asymmetry of the pelvis. Seventeen percent of the fish had an asymmetrical pelvis and, of these, 78% had greater expression on the left side; this directionality suggests a genetic influence. Females had consistently greater left-side asymmetry than did males. The incidence of total infection (all parasite species) in the largest adult fish (>60 mm body length) was greater in asymmetrical phenotypes, and this occurred in both sexes and for each parasite species (*Schistocephalus solidus*, *Cyathocephalus truncatus*, *Eustrongylides* spp.), even when multiple-species infections were excluded. Contrary to prediction, however, in juvenile fish (<20 mm) and yearlings (20–40 mm) but not subadults and adults (40–60 mm), asymmetrical phenotypes had significantly lower infection rates than symmetrical fish. This pattern occurred in both sexes, but the extent of the association varied over the 14 years of sampling. Consequently, if the directional asymmetry of the pelvis is under genetic control, asymmetry would be favoured during early ontogeny but selected against during the adult stages. The data support the hypothesis that asymmetry is a phenotypic signal of parasitism, but the unexpected bidirectionality of the association within a single population suggests increased complexity of the processes coupling asymmetry and genetic background.

Résumé : Les petits écarts de la symétrie bilatérale de certains traits morphologiques résultent de stress environnementaux et génétiques et peuvent être l'expression d'un bagage génétique inférieur. L'une des manifestations d'un génome inférieur est la résistance moins grande aux infections et, à cet égard, l'asymétrie peut être le reflet phénotypique de la sensibilité aux parasites. J'ai éprouvé cette hypothèse chez une population d'Épinoches à trois épines (*Gasterosteus aculeatus*) porteuse d'infections par des cestodes et des nématodes et affectée d'asymétries bilatérales pelviennes. Dix-sept pourcent des poissons de la population avaient un bassin asymétrique et, chez 78% de ceux-ci, l'asymétrie était plus prononcée du côté gauche; cette tendance indique probablement une influence génétique. L'asymétrie vers la gauche s'est avérée toujours plus fréquente chez les femelles que chez les mâles. Le parasitisme total (toutes les espèces de parasites) des poissons les plus gros (>60 mm de longueur) était plus fréquent chez les phénotypes asymétriques, un phénomène constaté aussi bien chez les mâles que chez les femelles et pour chaque espèce de parasite (*Schistocephalus solidus*, *Cyathocephalus truncatus*, *Eustrongylides* spp.), même en ne tenant pas compte des infections par plusieurs espèces de parasites. Cependant, contrairement aux prédictions, chez les poissons juvéniles (<20 mm) et les poissons de 1 an (20–40 mm), les taux d'infection étaient significativement plus faibles chez les phénotypes asymétriques que chez les poissons symétriques. Ce phénomène s'est vérifié chez les deux sexes, mais l'association s'est avérée variable au cours des 14 ans d'échantillonnage. Il semble donc que la tendance à l'asymétrie du bassin soit sous contrôle génétique et la sélection semble favoriser l'asymétrie au début du développement, mais la défavoriser chez les stades adultes. Les données supportent l'hypothèse selon laquelle l'asymétrie est une manifestation phénotypique du parasitisme, mais la tendance bidirectionnelle de cette association au sein d'une même population est indicatrice de la complexité des processus qui associent l'asymétrie et le bagage génétique.
[Traduit par la Rédaction]

Introduction

Bilateral asymmetry in morphological traits typically occurs at low frequency in many species. Some asymmetries are due to heritable factors that produce a left- or right-side bias during development (directional asymmetries) and result from adaptations and stochastic events in the genetic history of the species (Palmer et al. 1993). Most asymmetries originate from developmental instability and show a bell-shaped distribution with an equal left- and right-side bias; such a distribution characterizes fluctuating asymmetry and can be caused by environmental and physiological stress, chromosomal abnormalities, elevated homozygosity, or disruption of co-adapted gene complexes (Van Valen 1962; Bailit et al. 1970; Palmer and Strobeck 1986; Morbeck et al. 1991; Polak 1993; Alibert et al. 1994; Markow 1995). The asymmetries therefore represent an organism's inability to correct developmental errors that lead to departures from ideal symmetry and represent a phenotypic signal of weak genetic homeostasis (Soulé 1967; Livshits and Kobyliansky 1991; Møller 1992; Watson and Thornhill 1994). Because a weakened genetic background is

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itself associated with reduced resistance to pathogens and parasites (Wakelin 1978; Sage et al. 1986; O'Brien and Evermann 1988), it is reasonable to expect that asymmetry would also be a predictor of susceptibility to infection (Thornhill and Gangestaad 1993). There are only a few tests of this hypothesis and these are consistent with prediction (Polak 1993; Møller 1994). This observation is important, as it provides one of the explanations why phenotypes with asymmetry in epigamic traits are at a disadvantage during courtship (Møller 1992).

Stickleback (Gasterosteidae) occasionally exhibit bilateral asymmetry in the pelvic apparatus, usually in populations with a reduction in the size of the girdle (Nelson 1977; Reimchen 1980; Bell et al. 1985). When asymmetry occurs, greater expression tends to be more frequent on the left side in the threespine stickleback, *Gasterosteus aculeatus* (Reimchen 1980; Bell et al. 1985), and the distantly related brook stickleback, *Culaea inconstans* (Nelson 1977), but not in the nine-spined stickleback, *Pungitius pungitius* (Blouw and Boyd 1992). The heritability of pelvic asymmetry in *G. aculeatus* is not known, but directionality is generally assumed to have a genetic basis (Palmer et al. 1993). Pelvic-spine asymmetry is highly heritable in some members of the Gasterosteidae, such as *P. pungitius* (Blouw and Boyd 1992), but not in others, like *C. inconstans* (Nelson 1977).

As part of the investigations on insular lake populations of *G. aculeatus* from the Queen Charlotte Islands in western Canada (review in Reimchen 1994), a population was identified that exhibited bilateral variability in the pelvic girdle and variability in the number of spines (Moodie and Reimchen 1976). The population occurs in a remote, undisturbed lake and is unlikely to have incurred gene flow or other disruptions during historical times. Subsequent studies of the population showed that variability in the pelvic girdle was stable, marginally sexually dimorphic, and related to spatial differences in the predation regime (Reimchen 1980). Further work indicated infection by cestodes (Reimchen 1982) and nematodes (unpublished observations), the incidence of infection increasing in larger fish. This combination of pelvic-girdle variability and parasitism offered a novel opportunity to explore whether asymmetry is a predictor of parasitism in a natural population. I monitored the population for several years to test for this association and to examine the extent of temporal stability in any observed pattern. There are at least two mutually exclusive processes that could be involved if such an association is detected: (1) the parasites directly induce the asymmetries or (2) asymmetrical fish are more susceptible to parasites. The former is not probable in this study, as the pelvic bones are expressed in early ontogeny, prior to parasitic infection (Reimchen 1980, 1982). Evidence for the latter would support the use of directional asymmetry as a measure or signal of reduced genetic quality of an individual.

Parasite life histories

Three parasite species were recorded and these differ in their life history and host associations. The largest is *Schistocephalus solidus*. Initial hosts of this cestode are copepods, second hosts are stickleback, and definitive hosts are avian piscivores (Clarke 1954). Infections have been observed in all size classes of stickleback except fry (<15 mm standard length (SL)). When a juvenile stickleback (15–20 mm SL) consumes an infected copepod, the larval parasite (1–2 mm long) migrates through the intestinal wall into the coelom, where it develops into a large pleurocercoid (15–40 mm long). In this population, large pleurocercoids are rarely seen in fish less than 12 months of age, and the growth of the parasite typically requires an extended period in the host. Infected fish occur throughout the year, but the incidence of infection is greatest in summer months (Reimchen 1982). On maturation of the larvae, changes are induced in stickleback behaviour that increase the probability of capture by an avian piscivore (Arme and Owen 1967; Giles 1983; LoBue and Bell 1993).

A second cestode species infecting this stickleback population is *Cyathocephalus truncatus*. Initial hosts are amphipods, while the definitive hosts are fish (Vik 1958). In the fish, the larva attaches to the anterior region of the intestine near the pyloric sphincter and absorbs nutrients in the intestine. Initial infection occurs in stickleback near 35–40 mm SL, and maximum incidence of infection occurs during winter and early spring (Reimchen 1982). Infected fish typically have 2 or 3 larvae and exhibit increased consumption of benthic invertebrates relative to uninfected fish. Currently there is no information on the physiological effects of *C. truncatus* infection of stickleback, but it causes body lesions, emaciation, and mortality in salmonids (Vik 1954; Amin 1978).

A third parasite group examined was nematodes (*Eustrongylides* spp.) and these are found in the stomach and coelom or encysted in the muscle tissue. Initial hosts are aquatic oligochaetes, while stickleback are a secondary host and birds are the definitive hosts (Hoffman 1967). Infections were not common (<1%), but when present, they occurred predominantly on adult fish, which typically had 1 or 2 worms per fish (T.E. Reimchen, unpublished data). The physiological effects of this parasite on the fish host are not known but are potentially substantive given the large size of the nematode.

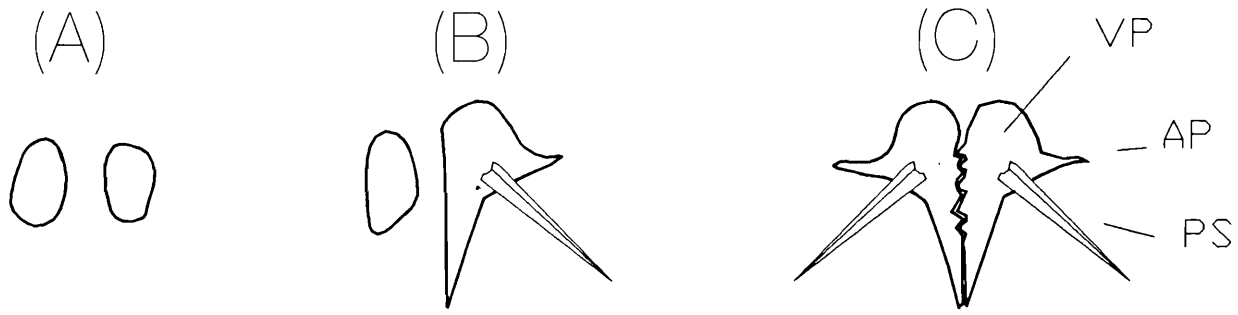
Study area and methods

The investigation was carried out at Boulton Lake, Graham Island, off the coast of central British Columbia, western Canada (the habitat is desribed in Reimchen 1980, 1982). The threespine stickleback was the only fish species in the lake in all years except one, when small numbers of Dolly Varden (*Salvelinus malma*) were present. The stickleback occupy all major habitats (littoral, limnetic, benthic, pelagic). Diving birds and macroinvertebrates are the most common piscivores. The stickleback exhibit morphological variability in the pelvic apparatus. This variability, which is continuous, includes full development with a ventral plate, an ascending process, and a spine on each side of the body, partial development with a ventral plate, an ascending process, and a spine on a single side, and highly reduced expression with two small ventral bones, which are remnants of the ventral plates (Fig. 1). I scored the fish into one of three classes: full pelvis, half pelvis, and no pelvis.

Samples were obtained in 1970, 1971, and 1975–1986. Sample sizes varied among years (range 782–2592) and a grand total of 20 380 fish were scored. I recorded SL, sex, number of lateral plates, number of dorsal spines, pelvic girdle class, and occurrence of each parasite species in all fish. In some individuals, the gonads were extensively atrophied and sex was not determined. From 1975 onwards, I also recorded the number of individuals of each parasite species. For *S. solidus*, I measured the contracted length of the largest pleurocercoid. The incidence of parasitism differed between the sexes (T.E. Reimchen, unpublished data), so in the following analyses the data are partitioned by sex.

I scored the occurrence of the pelvis on each side of the body and used signed values of right minus left (R–L) and absolute

Fig. 1. Schematic variability in pelvic girdle expression in threespine stickleback in Boulton Lake, Queen Charlotte Islands. Representative specimens are drawn but expression is highly variable. (A) No pelvis. (B) Half pelvis. (C) Full pelvis. AP, ascending process; VP, ventral plate; PS, pelvic spine.



values of |R−L| as the two major indices of asymmetry. Because there were only three categories (−1, 0, 1), skew and kurtosis were not calculated. For all analyses comparing asymmetrical and symmetrical groups for the presence or absence of parasites, I used the *G* test (log-likelihood) with sequential Bonferroni corrections for multiple tests (Rice 1989). When examining associations between symmetry and individual parasite species, I excluded all fish with multiple parasite infections in order to maintain the independence of any association between infection with individual parasite species and asymmetry. The numbers of parasites per individual for each group were compared using ANOVA and ANCOVA. These comparisons involved smaller data sets and gave the same results as the nonparametric tests. Thus, only presence/absence data are included here.

I looked for potential size-related patterns between parasitism and asymmetry. I partitioned the samples into size classes (usually 10-mm groups) separately for males and females and pooled the data across years. For each size class of fish, I used *G* tests to compare numbers of infected and non-infected fish for asymmetrical and symmetrical phenotypes. I also calculated and plotted frequency differences in the incidence of parasitism for asymmetrical and symmetrical classes (percent asymmetrical infected minus percent symmetrical infected). I also calculated the mean incidence of parasitism for asymmetrical and symmetrical classes, based on biannual samples over each of the 14 years of data. Means were determined for each size class with two methods, the first giving equal weight to each sample and the second weighting each sample according to the number of individuals. Unadjusted and adjusted means were compared using ANOVA, which gave similar results to the nonparametric tests in all cases and are not included here.

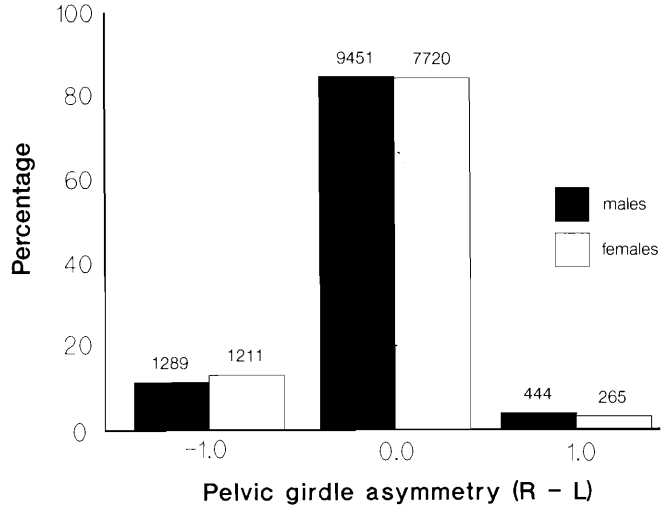
The stickleback pelvis is expressed early in ontogeny (near 15 mm SL), prior to the acquisition of parasites. However, it is conceivable that during growth, a parasitized fish with a full pelvis might lose a spine, perhaps because of the metabolic stresses resulting from the infection. Under these conditions, the frequency of asymmetrical pelvic girdles increases with age. I calculated a regression slope for pelvic asymmetry against body size (≥15 mm SL) for all parasitized fish, but this was not significantly different from zero (b = 0.00002, P = 0.40, t test), and I infer that the stress exerted on the host by the parasites does not influence expression of the pelvic girdle.

Results

General character asymmetry

The majority of fish were symmetrical, having either no pelvis (67.2%) or a complete pelvis with two equal halves (17%). The remainder (15.8%) were asymmetrical, usually with half a pelvis; of these individuals, approximately three-quarters (2501/3210) had the half pelvis on the left side (Fig. 2; mean (R−L) asymmetry = −0.085). The incidence of left-side asymmetry was slightly higher in females (males = −0.076, females = −0.103, G test on raw numbers = 28.8, 2 df, $P < 0.001$). This marginal directional asymmetry and the differences between the sexes occurred during most years (Fig. 3). Absolute |R−L| asymmetry was very similar between the sexes (males = 0.16, females = 0.16, G test on raw numbers = 0.9, 1 df, P = 0.34; Fig. 4).

Fig. 2. Frequencies of asymmetrical and symmetrical pelvic phenotypes. Numbers above the bars are sample sizes.



Incidence of parasitism and asymmetry

Males

Pooling all data for males yielded no significant relationship between the incidence of parasitism (all parasites) and pelvic asymmetry (N = 10226, symmetrical = 26.2%, asymmetrical = 25.5%, G = 0.42, P = 0.52). However, when the data were partitioned for parasite species (multiple species infections excluded), there were associations with asymmetry, but trends varied among species. Asymmetrical fish had a lower infection rate than symmetrical fish for *S. solidus* (N = 7822, symmetrical = 14.8%, asymmetrical = 10.8%, G = 13.8, P = 0.0002), a higher rate for *C. truncatus* (N = 5375, symmetrical = 20.2%, asymmetrical = 23.9%, G = 5.9, $P < 0.02$), and no difference for nematodes (N = 4397, symmetrical = 3.0%, asymmetrical = 4.1%, G= 2.2, P = 0.14).

In this population the incidence of infection increases in

Fig. 3. Yearly signed pelvic girdle asymmetry (mean ± 1 SE) in males and females. Numbers below the *x* axis are sample sizes.

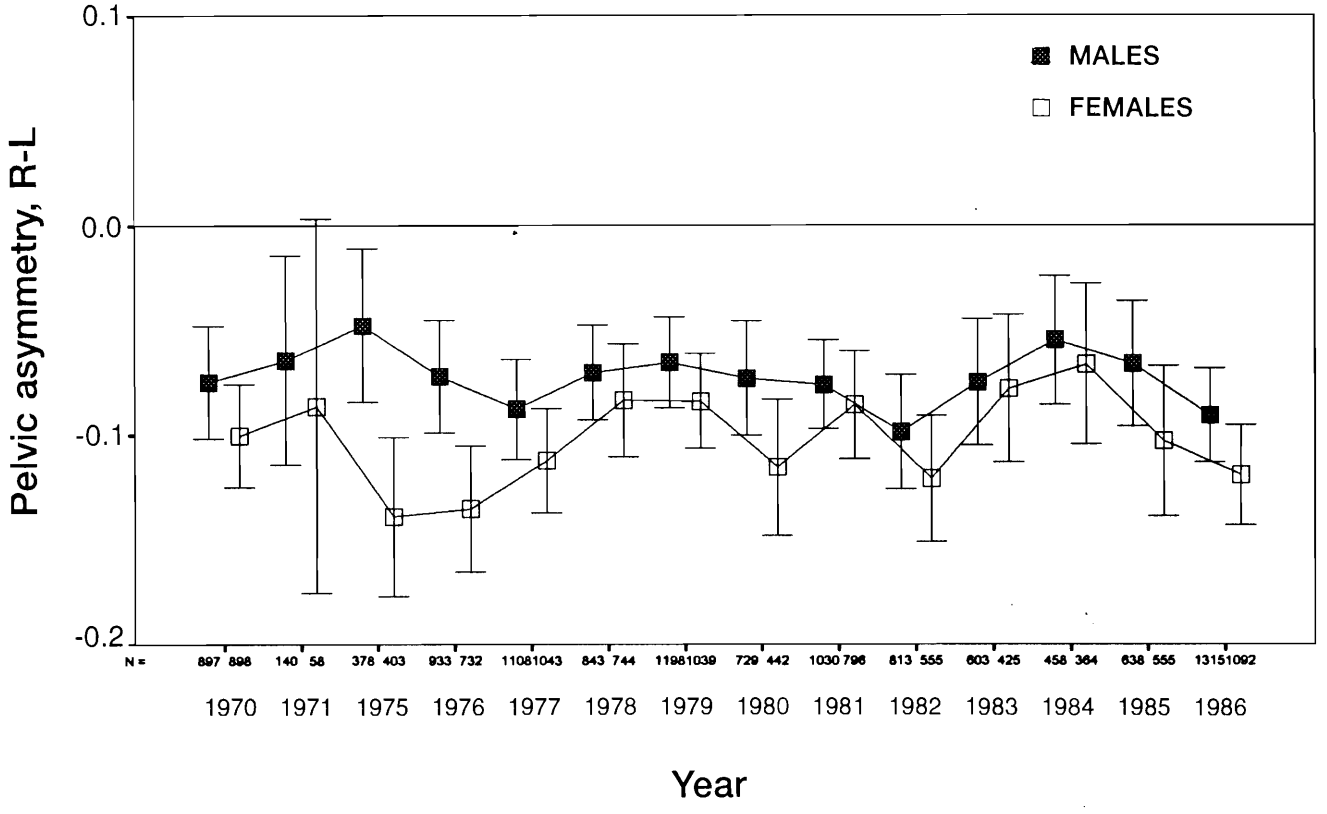
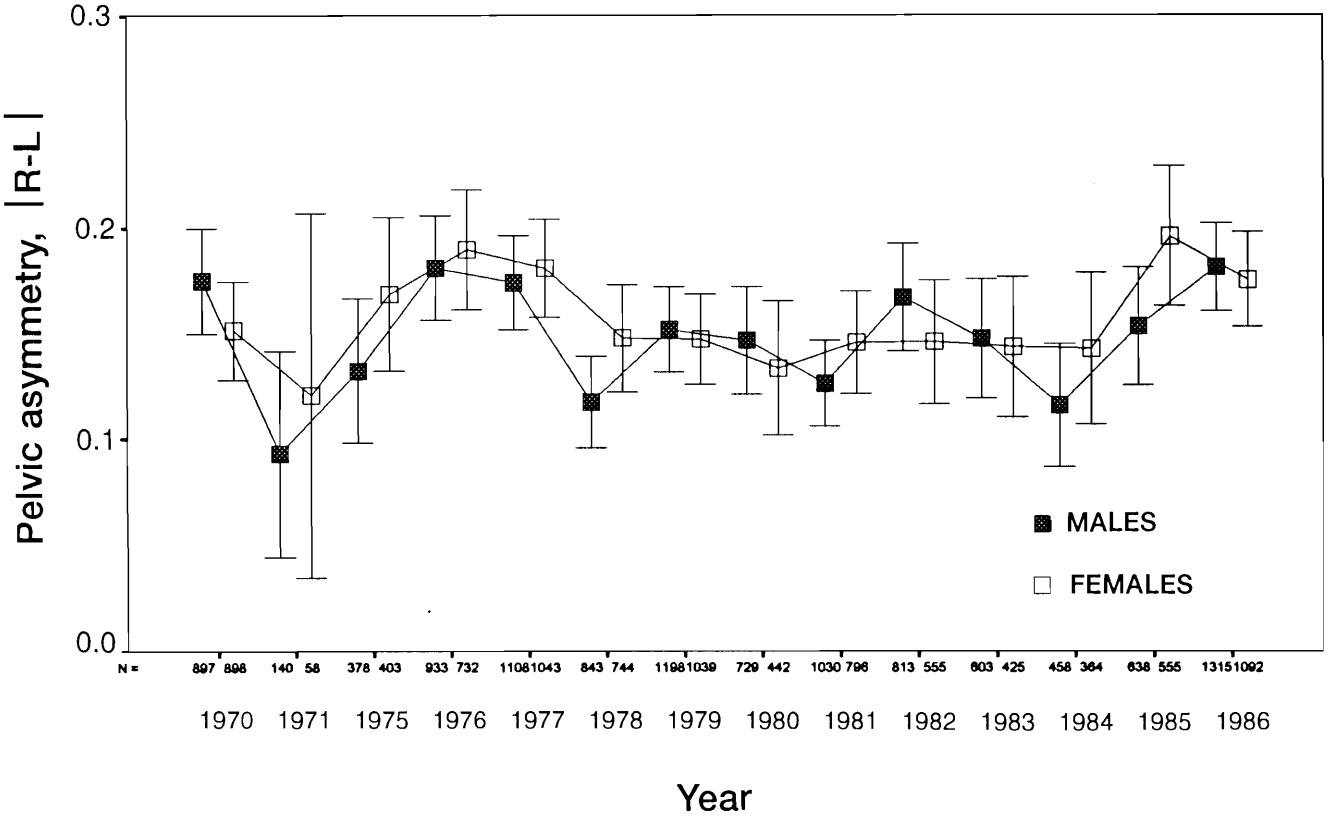


Fig. 4. Yearly absolute pelvic girdle asymmetry (mean ± 1 SE) in males and females. Numbers below the *x* axis are sample sizes.



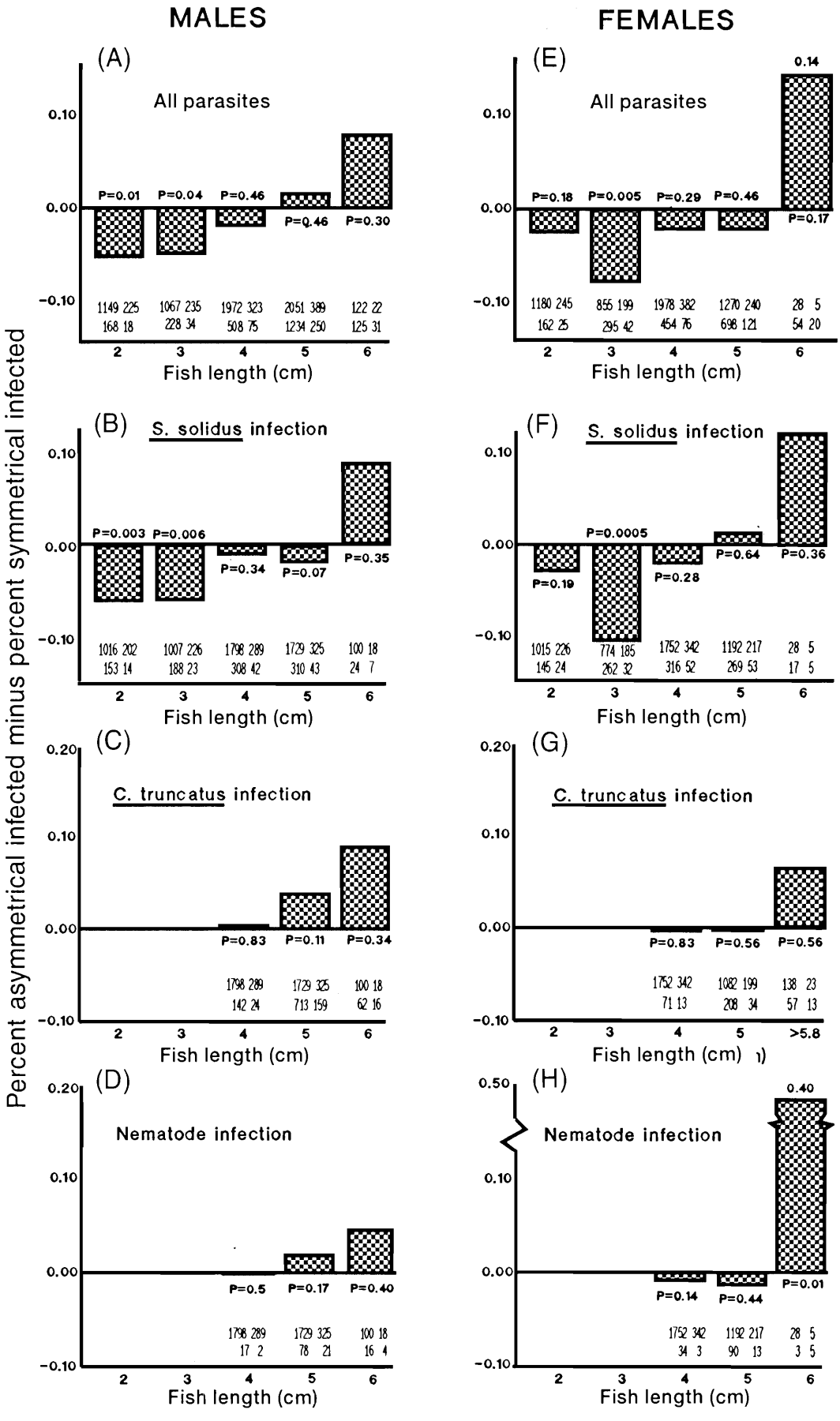


Fig. 5. Relative rates of parasite infection of asymmetrical and symmetrical pelvic girdle phenotypes plotted against size class in stickleback. (A) Males, all parasites. (B) Males, *S. solidus* infection. (C) Males, *C. truncatus* infection. (D) Males, nematode infections. (E) Females, all parasites. (F) Females, *S. solidus* infection. (G) Females, *C. truncatus* infection. (H) Females, nematode infections. The histograms show signed differences in frequency (percent asymmetrical infected minus percent symmetrical infected). Negative values indicate a deficiency of infections in asymmetrical fish and positive values indicate excess infections in asymmetrical fish. *G*-test probabilities are shown adjacent to each bar. Sample sizes for each comparison are given in an R × C table (row 1, noninfected; row 2, infected; column 1, symmetrical; column 2, asymmetrical).

older fish (Reimchen 1982), and it is possible that the observed relationship between infection with all parasites and asymmetry is an artifact of pooling size classes. When I partitioned the data for length groups, there was a deficiency of infections in small asymmetrical fish, equal infections among adults, and an excess in the largest asymmetrical fish (Fig. 5A). Log-likelihood tests reach statistical significance for the two groups with smaller body sizes.

Size-related analysis of the stickleback was also performed for each parasite species (multiple species infections excluded). This partitioning reduced sample sizes and the statistical confidence of each data set, but there were trends for each parasite species consistent with the larger grouped comparisons. For *S. solidus* (Fig. 5B), which infects all size classes of stickleback, younger fish (< 40 mm) exhibited a marked deficiency of infection in asymmetrical phenotypes relative to symmetrical fish. Among larger fish (40–60 mm), asymmetrical and symmetrical fish did not differ in infection rate, while in the largest adults (>60 mm), asymmetrical fish showed an elevated infection rate relative to symmetrical fish. For *C. truncatus* (Fig. 5C) and nematodes (Fig. 5D), which are not found in smaller fish (<40 mm), there was a marginal trend from equality of infection rate in the 40-mm group towards elevated infection rates in asymmetric phenotypes with an increase in the size of fish.

I looked for yearly trends in these ontogenetic differences and compared infection rates of asymmetrical and symmetrical fish in the two major size classes (small (<4.5 cm) and large (≥4.5 cm)) from 1970 to 1986. In the small fish, the data reflect predominantly the incidence of *S. solidus* infection, while in the large fish, infection is by all three parasites. Of 12 years of data that were available for small size classes, only for 1978 was there a significant difference between asymmetrical and symmetrical fish, using the Bonferroni correction, and in this case asymmetrical fish had a lower infection rate than symmetrical fish (Fig. 6). Heterogeneity was not significant among years ($G = 6.0$, 10 df), and pooling data showed a lower incidence of parasitism in asymmetrical individuals (symmetrical = 16.3%, asymmetrical = 10.9%, $G = 14.75$, $P = 0.0001$). In 10 of 12 years, there was the same signed difference, asymmetrical fish showing lower infection rate ($Z = -2.50$, $P < 0.02$; Wilcoxon's signed-rank test).

Among large males (Fig. 7), signed differences showed a deficiency of infection in asymmetrical fish in 5 years and an excess infection in 8 years ($Z = 1.78$, $P = 0.075$, Wilcoxon's signed-rank test). There was evidence of temporal grouping, as in 8 of the last 9 years of sampling, asymmetrical fish showed an elevated incidence of infection ($Z = 2.42$, $P = 0.015$). However, in only 1 of the years (1984) was the difference statistically significant. Heterogeneity among years was not significant ($G = 19.8$, 11 df, $0.1 < P < 0.25$), and subsequent pooling of the data indicated a slightly higher infection rate among asymmetrical fish (symmetrical = 32.9%, asymmetrical = 35.9%, $P = 0.07$).

Females

The overall data for females show a slightly lower infection rate (all species combined) in asymmetrical pelvic phenotypes than in symmetrical fish ($N = 8329$, symmetrical = 23.8%, asymmetrical = 21.0%, $G = 5.39$, $P = 0.02$). Partitioning for parasite species (multiple-species infections excluded) indicates that this trend only occurs for a single parasite species, *S. solidus*. Asymmetrical fish had a lower infection rate than symmetrical fish ($N = 6911$, symmetrical = 17.5%, asymmetrical = 14.5%, $G = 6.0$, $P = 0.014$). However, there were equal infection rates with *C. truncatus* ($N = 3932$, symmetrical = 10.2%, asymmetrical = 9.6%, $G = 0.2$, $P = 0.68$) and nematodes ($N = 3684$, symmetrical = 4.1%, asymmetrical = 3.6%, $G = 0.34$, $P = 0.56$).

Like the trends observed in males, there were ontogenetic shifts in relative infection rate, juvenile fish having a deficiency of infections in asymmetrical phenotypes, while the largest adults had excess infections (Fig. 5E). However, only the 3-cm size class yielded significance ($P = 0.005$), asymmetrical fish having a deficiency of infections. Partitioning the data for each parasite species also produced ontogenetic shifts. For *S. solidus*, asymmetrical fish had a deficiency of infections in juvenile fish but an excess in the largest fish (Fig. 5F). For *C. truncatus* and nematodes; infection rates were similar among smaller fish but progressively higher in asymmetrical phenotypes in the larger fish (Figs. 5G, 5H).

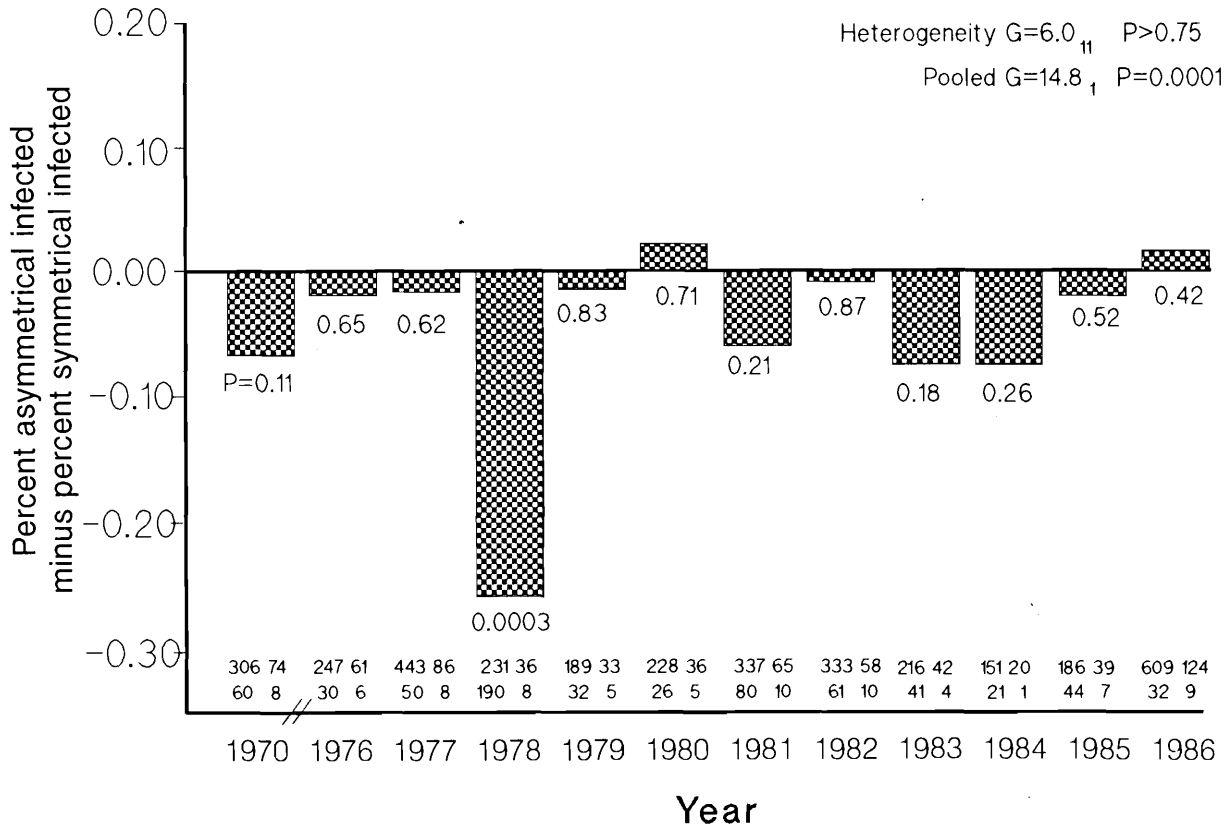
Yearly trends in relative infection rates in asymmetrical and symmetrical fish were examined for the two major size classes (small (<4.5 cm) and large (≥4.5 cm)). Of 12 years of data for small females, the data for 1981 and 1983 exhibited the greatest differences but in opposite directions (Fig. 8). However, these did not reach Bonferroni-corrected significance levels. Heterogeneity among years was significant ($G_H = 20.6$, 11 df, $P < 0.05$). Among large females (Fig. 9), there were no significant differences within any year, no heterogeneity among years ($G_H = 5.8$, 12 df, $P = 0.9$), and no overall pooled effect (symmetrical = 28.9, asymmetrical = 27, $G = 1.09$, $P = 0.30$).

To summarize, (*i*) during early ontogeny, asymmetrical fish of both sexes had lower infection rates than symmetrical fish, but in females there was significant heterogeneity among years, and (*ii*) the incidence of parasitism increased in larger fish and there was a general trend in both sexes and all parasite species towards a greater rate of increase in asymmetrical fish.

Discussion

Mean asymmetry of the pelvic girdle in Boulton Lake stickleback was directional rather than fluctuating, and its expression

Fig. 6. Relative rates of parasite infection of asymmetrical and symmetrical pelvic girdle phenotypes plotted against year for subadult male stickleback. For details see Fig. 5.



was increased on the left side in each year of observation. Left-side asymmetry associated with pelvic girdle reduction has also been reported in other *G. aculeatus* populations from Quebec (Edge and Coad 1983) and Alaska (Bell et al. 1985) and in brook stickleback from central Canada (Nelson 1977). In Alaskan populations, the directionality was more pronounced than at Boulton Lake, as more than 95% of the asymmetrical fish showed increased development on the left side. This directionality may have resulted from stochastic processes in the early history of the taxon, when genes influencing asymmetry went to fixation, leading to a "species-wide developmental bias" comparable to the side bias in many taxa of flatfish (Bell et al. 1985).

There is currently no evidence that pelvic girdle asymmetry in the threespine stickleback has any adaptive context. This is particularly evident in studies of Alaskan stickleback populations, where asymmetry involves differences in vestigial bones (Bell et al. 1985; Bell 1987). Pelvic girdle asymmetry of stickleback in Boulton Lake is slightly different in that it often comprises a normal spine and a normal ventral plate on one side. A half pelvis would be an advantage over no pelvis in courtship display (review in Wootton 1976) or during predation by vertebrate piscivores (Hoogland et al. 1957). Both of these factors could be operating in Boulton Lake stickleback (Reimchen 1980). However, a functional context to left-side bias in pelvic girdle expression would require asymmetry in courtship display of the stickleback or asymmetry in capture and handling by predators. Directional asymmetries in behaviour have been reported in vertebrates (Cole 1955; Leamy 1984; Clapham et al. 1995), but none have been described for stickleback (review in Bell and Foster 1994) or stickleback predators (Reimchen 1994).

On the assumption that there is genetic control of left-side asymmetry in the threespine stickleback and the genes have gone to fixation, it is puzzling that right-side asymmetry persists (5% in Alaskan *G. aculeatus* populations, 22% in Boulton Lake *G. aculeatus*, 45% in *C. inconstans*). Possibly, right-side asymmetry results from inferior developmental homeostasis (for example, see Møller 1994). If so, the excess parasitism that I observed in adult asymmetrical fish could be largely a consequence of an elevated incidence of parasitism of fish with right-side asymmetry rather than the genetically determined left-side asymmetry. I reanalyzed the data (from Fig. 5) and found that this was not the case: right-side asymmetrical fish had the same infection rates as left-side asymmetrical fish (adult males: left side = 42%, right side = 36.4%, G = 1.69, P = 0.19, N = 692; adult females: left side = 36.2%, right side = 37.7%, G = 0.05, P = 0.8, N = 386). I also partitioned the data for each parasite species, and in each case for both sexes, there were no significant differences between infection rates for left-side and right-side asymmetrical fish ($P > 0.2$ in all cases). Furthermore, the reversed pattern seen in juvenile fish, that is, a deficiency of infections in asymmetrical individuals, also occurred for both left-side and right-side asymmetrical fish.

The major focus of this study was to test whether asymmetric phenotypes had elevated infection rates relative to symmetric individuals. This was confirmed for both sexes

Fig. 7. Relative rates of parasite infection of asymmetrical and symmetrical pelvic phenotypes plotted against year for adult male stickleback. For details see Fig. 5.

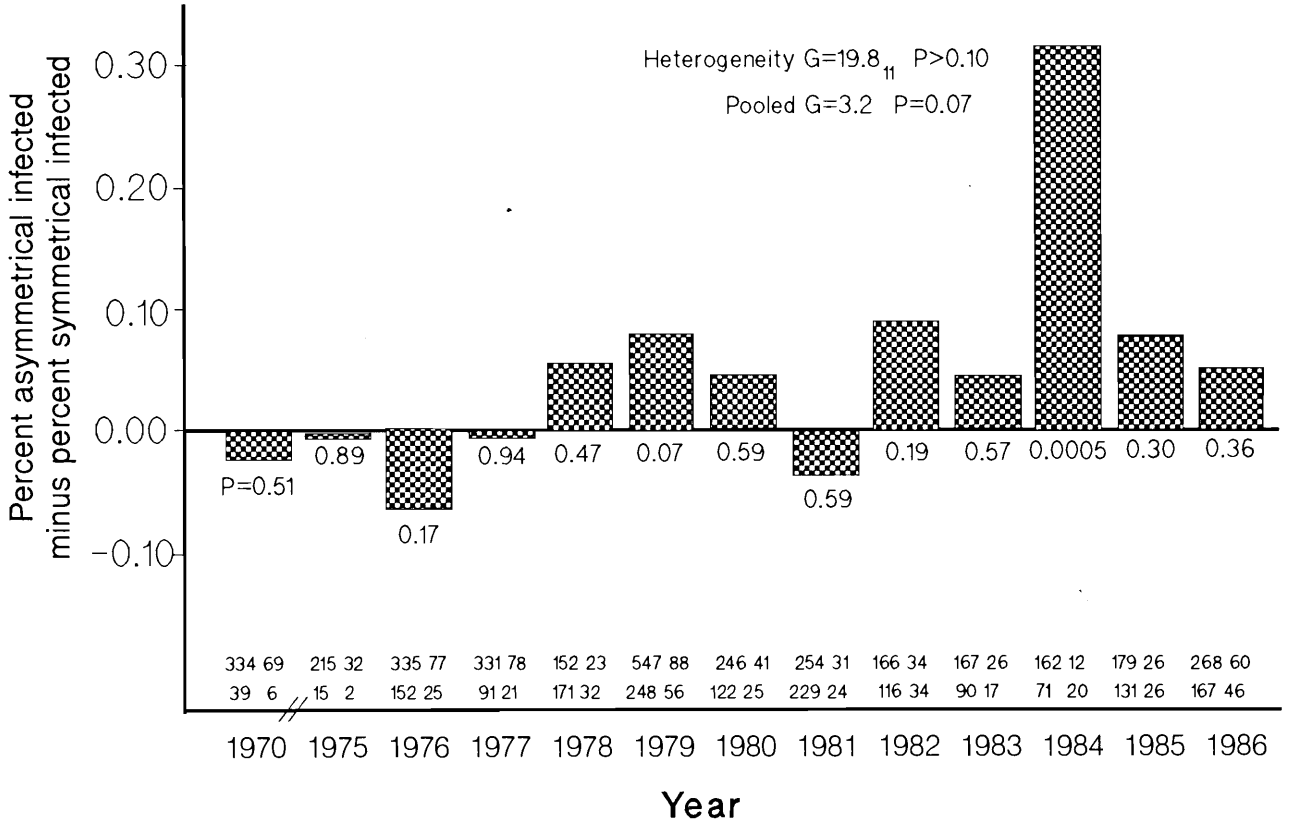


Fig. 8. Relative rates of parasite infection of asymmetrical and symmetrical pelvic phenotypes plotted against year for subadult female stickleback. For details see Fig. 5.

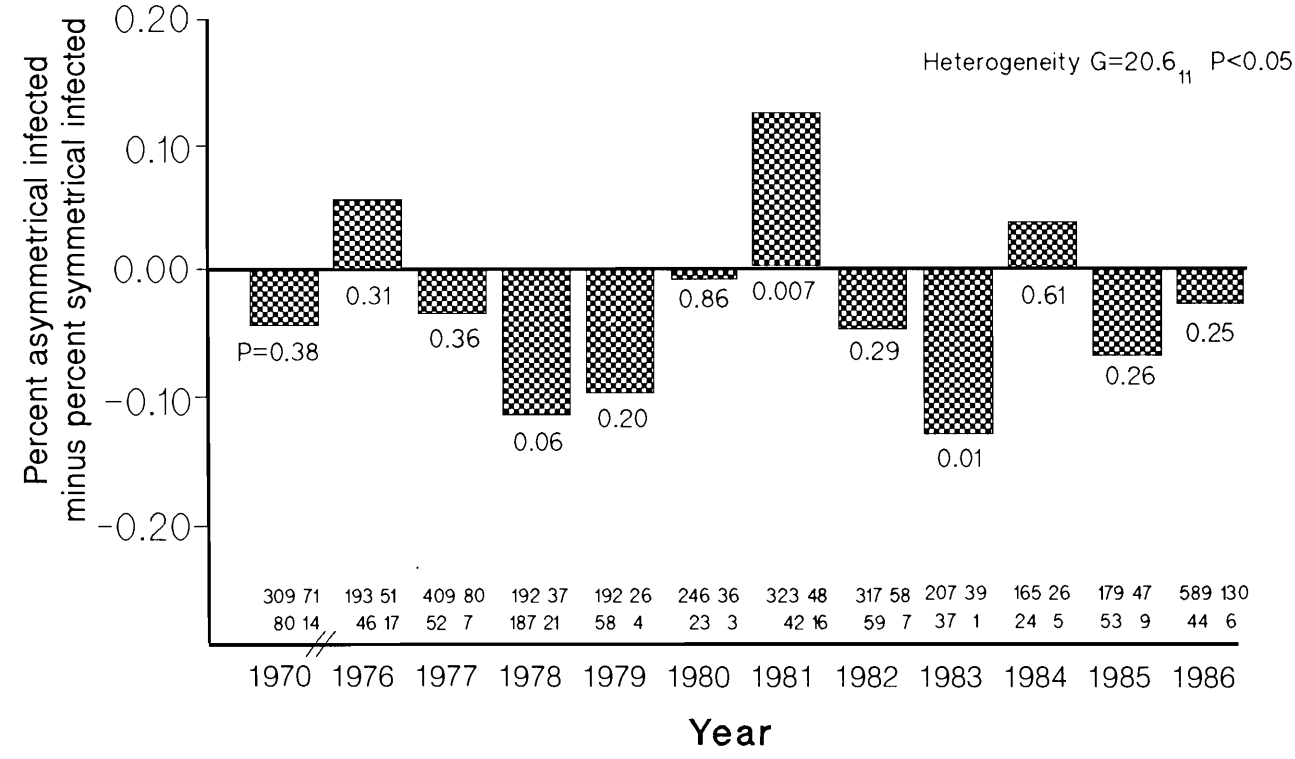
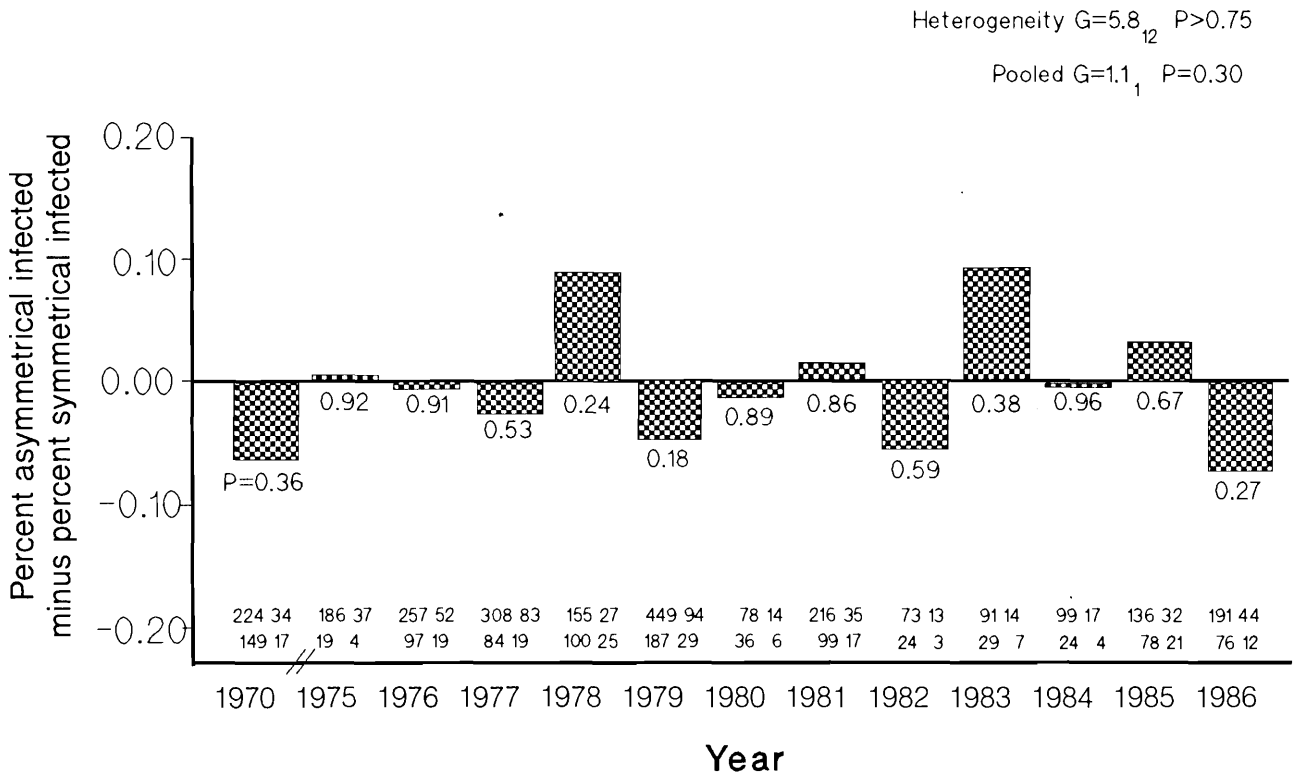


Fig. 9. Relative rates of parasite infection of asymmetrical and symmetrical pelvic phenotypes plotted against year for adult female stickleback. For details see Fig. 5.



but only in larger fish. Elevated levels of infection of asymmetrical phenotypes have been previously reported in birds (Møller 1992) and insects (Polak 1993). These studies differ from the present one in that the parasites directly induce the asymmetries in the bird and insect hosts. In this context, parasitism is one of many environmental stresses that lead to developmental errors and asymmetry (see the recent review in Markow 1995). In the current study, asymmetry occurs prior to acquisition of parasites, therefore the elevated incidence of infection of asymmetrical fish in the adult stages must be caused by the presence of some physiological or life-history attribute prior to the adult stage that leads to an increased likelihood of infection. If it is a physiological attribute, such as an inferior capacity to resist infection, the finding is consistent with the postulated association between reduced immunocompetence and asymmetry (Thornhill and Gangestaad 1993). That the relative incidence of infection of asymmetrical phenotypes increased with the size/age of fish of both sexes and each parasite species suggests that over the life history of the fish, there is a persistent difference in the capacity to avoid or reject new infections.

One of the unexpected and contradictory results emerging from this study was the reduced incidence of *S. solidus* infection in juvenile asymmetrical fish. The replicated trend in both sexes and to a lesser extent among years suggests that this is biologically meaningful. Such an effect runs counter to predicted associations and appears to weaken the general conclusion that pelvic girdle asymmetry can be a meaningful signal of an inferior genome. Under some exceptional conditions, infection may be an advantage for the host, for example, when one species of parasite reduces infection rates by more injurious species (see Holmes 1983; Curio 1988). If applicable here, this would imply that *S. solidus* infections in symmetrical juveniles reduce the incidence and severity of infections by *C. truncatus* and *Eustrongylides* spp. in older age-classes. While this may be occurring, it is unlikely to explain the current data, as *S. solidus* infections result in an increase in oxygen requirements, foraging requirements, and predation risk and a reduction in the reproductive capability of the host (Clarke 1955; Lester 1971; Giles 1983; Godin and Sproule 1988; LoBue and Bell 1993); these findings justify the assumption that the presence of *S. solidus* is a disadvantage to the fish and that symmetrical juveniles have a lowered fitness level relative to asymmetrical juveniles for this selective regime. Because pelvic girdle asymmetry must be a consequence of either a weak genetic background and developmental instability or a genetically controlled left-side bias in development, the potential consequence of a reduced incidence of infection in asymmetrical fish is selection for asymmetry. There is a precedent for this suggestion, as the results of recent studies on bilateral variability in the number of bony lateral plates in adult threespine stickleback suggest that asymmetric males are more successful than symmetric males during nest defense (Moodie and Moodie 1996).

Immunological responses to and defenses against infection are accentuated by enzyme heterozygosity (Tooby 1982; O'Brien and Evermann 1988; Thornhill and Gangestad 1993; Mitton 1995) and this might contribute to some of the differential infections of the asymmetrical pelvic girdle phenotypes. The genetics of pelvic girdle expression in Boulton Lake stickleback are unknown, but for the present purposes assume that fish with half a pelvis are more heterozygous than those

with a complete pelvis or no pelvis. If such heterozygosity for a structural character was correlated with heterozygosity for enzymes, this could account for the reduced infection rates in asymmetrical juveniles. Yet if this were true, it would require a reversal with age of the fish, as there was an elevated incidence of infection in asymmetrical adults.

An alternative interpretation of the ontogenetic shift in relative rates of parasitism in symmetrical and asymmetrical phenotypes is that the phenotypes differ in dietary niche. Seasonal and ontogenetic differences in rates of parasitism in this population are broadly correlated with seasonal and ontogenetic shifts in diet (Reimchen 1982). For example, the increased occurrence of *C. truncatus* infections in winter coincides with increased winter consumption of amphipods, the initial hosts of this parasite. Although stickleback require about 12 months to reach 35–40 mm SL (Reimchen 1992), first infections in stickleback occur near 40 mm SL, coincident with the first occurrence of amphipods in the diet. Furthermore, fish with greater numbers of amphipods in the stomach also had a greater intensity of infections (Reimchen 1982). Consequently, it is reasonable to suggest that differences in microhabitat of the fish alter the probability of infection. If there were niche differences between symmetrical and asymmetrical fish, possibly due to competitive interactions, this could result in different probabilities of encounters with infected primary hosts. Such a suggestion is plausible, since pelvic girdle phenotypes among young age-classes differ predictably in their distribution in the lake. Those without a pelvis occur at higher frequencies near shore, and those with a full or half pelvis occur at higher frequencies in open-water habitats (Reimchen 1980). This difference occurs in both males and females and is most accentuated in juveniles (<20 mm SL). Such habitat differences among phenotypes provide the fish with different prey choices, ranging from a predominance of benthos in littoral habitats to plankton in open water as well as to fine-scale spatial differences in prey taxa within each of these habitats. The reduced rates of infection in juvenile fish with *S. solidus* in asymmetrical phenotypes could simply reflect a lowered probability of encounters with infected copepods. Diet-induced effects could also account for the general equality of infection with cestodes and nematodes among asymmetrical and symmetrical phenotypes in fish larger than 40 mm SL, as there appears to be random spatial distribution of the phenotypes in these size classes (Reimchen 1980). However, the shift towards accentuated infection of asymmetric phenotypes in the largest fish cannot be explained by diet without postulating either a secondary subdivision of niches in the opposite directions from that observed in juveniles, for which there is no evidence, or an increased susceptibility to infection. The latter would be predicted from general theory coupling asymmetry with reduced immunocompetence.

In conclusion, the prediction of an increased incidence of parasitism in asymmetrical fish is supported in this study, at least for adult size classes. The similar trends for each of the three parasite species and each sex provide confidence that the observed effects are biologically meaningful. There is growing evidence from different taxa that during courtship, symmetry in sexual traits is related to attractiveness and is a useful signal of the genetic quality of the mate (Møller 1990; Polak 1993; Thornhill and Gangestad 1993; review in Watson and Thornhill 1994). Because stickleback use visual cues during courtship (Wootton 1976), it seems possible that pelvic girdle symmetry could function as an epigamic trait and be a reliable signal of genetic quality. Whether the differences in rates of parasitism are due to immunological effects, niche differentiation, or combinations thereof is currently under investigation.

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