THE ADAPTIVE SIGNIFICANCE OF DORSAL SPINE VARIATION IN THE FOURSPINE STICKLEBACK, *APELTES QUADRACUS*. IV. PHENOTYPIC COVARIATION WITH CLOSELY RELATED SPECIES

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SUMMARY

The fourspine stickleback (*Apeltes quadracus*) and ninespine stickleback (*Pungitius pungitius*) have similar ecologies, they often coexist, and they share parallel polymorphisms for the number of dorsal spines. Spine number is positively correlated between them for 86 sites in eastern Canada. Dorsal spine length is positively correlated with spine number within each species, and spine length is positively correlated between them. Spine length for both species is also positively correlated with the presence of predatory fishes and negatively correlated with vegetation cover. Finally, spine number for both *Apeltes* and *Pungitius* is lower where they coexist with a new species of stickleback (*Gasterosteus* sp.) which is found only in environments where predation risk is low. These patterns of covariation within and between species are evidence for natural selection, and they suggest that predators are a selective agent favouring the higher-spined morphs. We discuss these results with respect to the idea that geographic variation in *Pungitius* reflects historical processes of isolation and differentiation during the Pleistocene era.

1. INTRODUCTION

A powerful method for detecting the action of natural selection is to search for covariation among homologous polymorphic traits in closely related and ecologically similar species where they coexist (Clarke, 1975; Koehn and Mitton, 1972; Turner 1977). Our research is aimed at identifying the adaptive significance of a polymorphism for the number of dorsal spines in *Apeltes quadracus*, the fourspine stickleback (Blouw, 1982; Blouw and Hagen, 1981, 1984a, b, c; Hagen and Blouw, 1983). As one of several approaches to this problem, we investigate covariation between the phenotypes of *Apeltes* and those of *Pungitius pungitius*, the ninespine stickleback, which shares with *Apeltes* a polymorphism for the number of dorsal spines. We also investigate covariation between these species and the distribution of a new species of stickleback in Nova Scotia, Canada (Hagen, Blouw and Armstrong, in preparation).

Spine number in *Apeltes* is highly heritable ($h^2 \approx 0.61$; Hagen and Blouw, 1983). The dorsal spines (fig. 1) vary in number from one to seven, but these extremes are very rare. A geographic survey of 570 sites in the Maritime Provinces of Canada (Blouw and Hagen, 1984a) shows that nearly all populations are polymorphic. The four- and five-spined morphs are the

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FIG. 1. Lateral views of *Apeltes quadracus* (above) and *Pungitius pungitius* (below). Dorsal spine number is polymorphic in both species; shown are the five-spined morph of *Apeltes* and the nine-spined morph of *Pungitius*.

most common, with the three- and six-spined less common and rare. Morph frequencies are highly differentiated geographically, with areas of relatively constant frequencies, gentle clines, steep clines, and remarkably abrupt changes we call "intrusions", where frequencies at some sites differ greatly from those at a larger number of surrounding sites (Blouw and Hagen, 1984a). One of these intrusions, at Louisbourg Harbour, Nova Scotia, is of particular interest with respect to the covariation between *Apeltes* and *Pungitius* (see below).

The geographic variation in spine number in *Apeltes* is correlated with environmental differences among sites (Blouw and Hagen, 1984b). The correlations suggest that predatory fishes act as selective agents favouring the higher-spined morphs. Indeed, populations with higher mean spine number also have longer spines and larger mean body size than populations with fewer spines (Blouw and Hagen, 1984c). Laboratory and field experiments with predators (four fish and one bird species) show that the spine morphs are differentially susceptible to predation. However the higher-spined morphs are not always favoured, and vegetation plays a modifying role (Blouw and Hagen, 1984c). Thus the relationship between spine number and predation is complex.

The dorsal spines of *Pungitius* (fig. 1) are shorter than those of *Apeltes*, they are more uniform in length and more numerous, but as in *Apeltes*, they are set at acute angles to the vertebral body axis. The two species are similar in size, prefer vegetated areas, nest in vegetation, are cryptically coloured with similar tones outside the breeding season, the breeding colours of males emphasize the pelvic spines, the breeding seasons overlap, they often reside in the same places outside the breeding season, and the diets are similar (Wootton, 1976; Worgan and Fitzgerald, 1981).

2. MATERIALS AND METHODS

We collected *Pungitius* with *Apeltes* at 86 sites. Sampling and scoring methods for *Pungitius* are as described for *Apeltes* (Blouw and Hagen, 1981, 1984c) except that the longest of the first three dorsal spines was measured for length. Dorsal spines in *Pungitius* vary from 6 to 13, but only the eight- to eleven-spined morphs are common. We examined homogeneity of morph frequencies between the sexes and among adults and juveniles; the results (table 1) show that, as in *Apeltes* (Blouw and Hagen, 1981), morph frequencies are homogeneous for both variables. The sexes and size classes are therefore pooled to estimate morph frequencies at each site.

3. RESULTS

(i) Covariation with *Pungitius*

Sampling sites and scores for *Apeltes* and *Pungitius* are shown in table 2. The samples are from the entire area of our detailed geographic survey (Blouw and Hagen 1984a), and they cover the full range of dorsal spine variation in *Apeltes*. Spine number in *Pungitius*, like that of *Apeltes*, is highly differentiated among sites (table 2). Thus there is ample geographic variation to test for covariation between them.

The correlation between dorsal spine number in *Apeltes* and *Pungitius* (fig. 2) is positive and highly significant ($r=0.45$; $P<0.001$). However, the covariation breaks down at two sites (lower right corner, fig. 2). These are at the intrusion for high spine number of *Apeltes* at Louisbourg Harbour (sites 324 and 325, table 2). Thus the agent responsible for high spine number in *Apeltes* at Louisbourg Harbour does not produce a parallel response in *Pungitius*, suggesting that some unique process occurs there.

Earlier studies suggest that predators are selective agents on the spines of *Apeltes* (Blouw and Hagen, 1984b, c). If the covariation between *Apeltes* and *Pungitius* is due to selective predation, we predict that spine length in *Pungitius* should be greater where spine number is higher, as is found in *Apeltes* (Blouw and Hagen, 1984c). We scored 43 samples of *Pungitius* for spine length (table 2). To control for size differences among populations, we standardised spine length to a common body length of 33 mm for each site using intrapopulation regression equations of spine length on body length. Spine length and spine number are positively correlated among populations ($r=0.41$; $P<0.01$). The 43 samples used to score spine length for *Pungitius* were also used to score spine length for *Apeltes* (table 2) and, as expected, standardised spine length is positively correlated between them ($r=0.64$, $P<0.001$). Thus *Apeltes* and *Pungitius* vary in parallel for both spine number and spine length, and the pattern is consistent with the hypothesis that predators are selective agents favouring the higher-spined morphs.

It is clearly of interest to see if spine length is in fact greater at sites where predators are present. Samples were scored as having predators using the methods of Blouw and Hagen (1984b). Predators were shown to be present at only 7 of the 43 sites during our survey (however this does not necessarily mean that the other 36 sites do not have predators—see Blouw and Hagen, 1984b for limitations of predator sampling). Because of these

TABLE 1
Tests for homogeneity of morph frequencies between the sexes (A) and age groups (B) of Pungitius pungitius, the ninespine stickleback

A. Site ^a	♂ Spine Morph					♀ Spine Morph					χ^2	P
	8	9	10	11		8	9	10	11			
Buctouche River 7 N.B.	—	14	24	3		—	75	157	30		0.94	>0.50
Trout River P.E.I.	14	162	122	3		10	107	113	6		5.47	>0.10
Daigle Inlet 17 N.B.	—	54	59	—		—	32	37	—		0.03	>0.75
Daigle Inlet 14 N.B.	5	50	60	7		3	59	66	3		2.81	>0.25
B												
	Sexed (Adult)					Unsexed (Juvenile)						
Daigle Inlet 14 N.B.	8	109	126	10		8	127	121	6		2.32	>0.50

^a N.B. = Province of New Brunswick, P.E.I. = Province of Prince Edward Island.

TABLE 2

Sampling sites, and scores of dorsal spine number and dorsal spine length for *Apeltes* and *Pungitius* at 86 sites in the Maritime Provinces of Canada

No. ^a	Site Name	Province ^b	Apeltes				Pungitius			
			Lat. ^c	Long. ^c	N	Spines ^d	Length ^e	N	Spines ^d	Length ^f
34	French Fort Brook	NB	47-018	65-554	727	4-66	—	185	9-64	—
41	Napan River 1	NB	47-060	65-330	925	4-71	—	34	9-68	—
60	Kouchibouguac River 3	NB	46-822	64-964	232	4-35	—	86	9-36	—
67	Daigle Inlet 17	NB	46-723	64-913	132	4-29	3-85	192	9-54	2-06
73	Daigle Inlet 1	NB	46-706	64-897	842	4-29	3-51(101)	40	9-53	1-73
74	Daigle Inlet 14	NB	46-695	64-909	191	4-31	—	515	9-51	—
139	Robinson Creek 1	NB	46-172	63-959	1112	4-10	—	66	9-68	—
141	Spence Settlement	NB	46-149	63-902	167	4-08	—	159	9-96	—
148	Peacock Cove 3	NB	46-148	63-867	760	4-40	3-61(87)	60	10-08	1-77
149	Peacock Cove 1	NB	46-145	63-861	1034	4-39	—	32	9-59	—
152	Peacock Cove 4	NB	46-151	68-857	122	4-43	—	119	9-91	—
155	McKay Brook 2	NB	46-095	63-789	1041	4-30	3-27(134)	78	9-86	1-56
156	McKay Brook 1	NB	46-099	63-792	63	4-35	—	50	9-84	—
157	Barry Brook 1	NB	46-066	63-886	67	4-28	3-43	110	9-93	1-73
161	Fort Monckton Point	NB	46-040	64-065	1395	4-56	—	79	9-77	—
165	Big Cove 2	NB	45-998	64-070	171	4-41	—	36	10-08	—
166	Big Cove 1	NB	45-995	64-058	1082	4-53	—	68	9-75	—
171	Tidnish River 3	NS	45-947	64-066	112	4-39	—	30	10-20	—
173	Tidnish Dock	NS	45-997	64-009	1135	4-56	3-64(232)	65	10-17	1-91
176	Shinimicas River 1	NS	45-936	63-855	41	4-17	3-41	59	9-71	1-80
179	Linden 1	NS	45-890	63-807	86	4-10	—	37	9-70	—
199	John Bay 1	NS	45-767	63-059	103	4-14	3-24	43	9-79	1-69
203	Carbou Harbour 1	NS	45-731	62-745	108	4-06	—	20	9-80	—
215	Merigomish Harbour 3	NS	45-629	62-432	45	4-07	—	55	9-62	—
217	Malignant Cove	NS	45-787	62-079	82	3-94	2-29	58	9-62	1-67
218	Lakevale	NS	45-781	61-907	101	3-99	2-74	58	9-57	1-61
219	West Lakevale	NS	45-767	61-912	59	4-05	—	59	9-32	—
221	Lanark	NS	45-636	61-950	78	4-03	—	36	9-06	—

TABLE 2—continued

No. ^a	Site Name	Province ^b	Lat. ^c	Long. ^c	Apeltes			Pungitius		
					N	Spines ^d	Length ^e	N	Spines ^d	Length ^f
229	Bayfield	NS	45-631	61-733	86	4-01	—	67	9-37	—
234	Havre Boucher	NS	45-674	61-567	41	4-07	—	39	9-51	—
238	McKay Point	NS	45-907	61-491	99	4-05	—	31	9-45	—
241	Mabou Harbour Mouth	NS	46-087	61-452	50	4-02	—	21	9-57	—
242	Mabou Harbour 2	NS	46-060	61-428	56	3-91	2-49	30	9-27	1-71
248	East Margaree	NS	46-394	61-076	61	4-05	—	62	9-26	—
257	Freshwater Lake	NS	46-644	60-397	60	4-33	2-39	40	9-15	1-44
258	Ingonish Harbour 2	NS	46-630	60-420	101	4-08	—	60	9-53	—
262	New Campbellton	NS	46-290	60-428	58	3-98	2-72	38	8-89	1-56
264	Crescent Grove	NS	46-114	60-719	51	4-02	3-23	60	9-00	1-60
266	Middle River	NS	46-083	60-900	23	4-17	—	62	9-08	—
270	Iron Mines	NS	45-932	61-080	104	3-88	2-90	62	9-26	1-89
271	South Side Whycocomagh	NS	45-938	61-054	64	3-84	2-64	77	9-17	1-56
276	Gillis Point 1	NS	46-023	60-786	115	4-02	—	49	9-04	—
279	Ottawa Brook	NS	45-933	60-936	16	3-63	—	62	8-98	—
280	Whycocomagh Portage	NS	45-943	60-991	84	3-64	2-74	55	9-09	1-57
284	Gillis Cove	NS	45-912	61-051	53	3-49	2-97	77	9-03	1-45
287	River Denys	NS	45-849	61-124	49	3-61	2-66	59	9-08	1-55
292	McRae's Point	NS	45-746	60-840	40	3-85	2-62	43	9-33	1-62
297	McNab Cove	NS	45-730	60-721	59	3-93	2-63	57	9-32	1-45
300	East Bay 1	NS	46-015	60-376	50	4-16	2-57	38	9-32	1-56
301	East Bay 2	NS	46-023	60-363	39	4-10	—	65	9-18	—
303	Castle Bay	NS	45-934	60-637	43	3-98	2-73	58	9-26	1-53
307	Piper Cove	NS	45-938	60-754	67	4-04	—	45	9-07	—
308	Grand Narrows	NS	45-952	60-789	60	3-72	2-58	81	9-19	1-64
309	Christmas Island Inlet	NS	45-962	60-767	103	3-90	2-64	38	8-87	1-46
312	Saunders Cove	NS	46-242	60-358	63	4-00	3-14	86	8-98	1-69
313	Point Aconi 1	NS	46-323	60-319	64	3-98	—	50	8-92	—
320	Mira River 2	NS	45-825	60-285	39	4-17	—	21	9-52	—
322	Baleine	NS	45-950	59-824	44	4-00	2-78	54	9-19	1-52

324	Louisbourg	NS	45-921	59-963	223	4-67	3-44	26	9-00	1-66
325	Fortress Louisbourg	NS	45-896	59-991	47	4-85	3-27	34	8-91	1-51
329	Gabarus Lake	NS	45-812	60-229	64	4-09	2-72	45	9-07	1-32
330	Fourchu	NS	45-714	60-253	60	3-93	2-76	50	8-96	1-59
331	Kempt Point	NS	45-628	60-515	49	3-94	2-48	49	9-14	1-35
332	Grand River	NS	45-620	60-636	85	4-12	—	47	9-04	—
333	L'Ardoise	NS	45-598	60-745	40	4-30	—	22	9-55	—
342	Melford	NS	45-545	61-329	83	4-02	—	31	9-52	—
343	Eddy Point	NS	45-517	61-244	110	4-01	—	81	9-54	—
346	Moose Point	NS	45-403	61-427	103	4-14	—	53	9-57	—
364	New Harbour River 2	NS	45-210	61-509	147	3-97	2-84	48	9-35	1-44
381	Moser River	NS	44-968	62-252	82	4-06	3-61	46	9-48	1-87
396	Clam Bay	NS	44-729	62-907	117	3-85	2-20	52	9-08	1-54
399	West Jeddore	NS	44-725	63-036	47	4-19	3-66	65	9-23	1-86
414	Peggy's Cove	NS	44-498	63-897	135	3-99	2-49	47	9-13	1-44
417	Chester Basin	NS	44-567	64-298	38	4-08	—	22	9-32	—
436	Green Harbour 1	NS	43-740	65-140	105	4-18	3-16	53	9-02	1-80
438	Negro Harbour 1	NS	43-568	65-411	68	4-28	3-05	31	9-48	1-94
439	Port Clyde	NS	43-598	65-462	55	4-11	—	42	9-26	—
441	Upper Port LaTour	NS	43-511	65-486	94	4-17	3-20	52	9-12	1-90
444	Doctors Cove	NS	43-518	65-622	76	4-08	—	69	9-30	—
454	Saultnierville	NS	44-258	66-122	122	3-96	2-82	47	9-17	1-83
457	Annapolis River 5	NS	44-902	65-117	39	3-90	2-43	35	9-63	1-65
468	Gays River	NS	45-026	63-355	67	4-06	—	27	9-33	—
469	Parrsboro River	NS	45-415	64-303	73	4-01	—	46	8-98	—
485	Newfoundland Creek	NB	45-628	64-805	344	3-99	2-69(69)	49	9-37	1-46
555	Trout River	PEI	46-694	64-149	76	4-32	—	544	9-43	—
560	Trout River 2	PEI	46-408	63-417	352	4-47	—	41	9-20	—

^a Site numbers correspond to those in Blouw (1982) and Blouw and Hagen (1984a, b, c).

^b For provinces; NB = New Brunswick, NS = Nova Scotia, PEI = Prince Edward Island.

^c Latitude and Longitude are given in decimal degrees north and west.

^d Mean spine number; morph frequencies are given in Blouw (1982).

^e Dorsal spine length standardised to a common body length of 29 mm for each site (see text). Sample sizes for spine length estimates are shown in parentheses if different from the sample size used to estimate mean spine number, shown in the column headed by N.

^f Dorsal spine length standardised to a common body length of 33 mm for each site (see text).

FIG. 2. The relationship between mean spine number in *Apeltes* and mean spine number in *Pungitius*. The two points at bottom right are from sites at Louisbourg Harbour, Nova Scotia.

highly unequal sample sizes the median test was applied, and the contingency tables (table 3) were analysed by Fisher's exact test (Zar, 1974). The median test is only about 64 per cent as powerful as analysis of variance so evaluation of the null hypothesis of equal spine lengths is conservative. Yet the null hypothesis is strongly rejected for both *Apeltes* and *Pungitius* (table 3); dorsal spines are longer where predators occur.

Previous studies also show that spine number in *Apeltes* is higher at sites with aquatic grasses and lower at sites with filamentous algae (Blouw and Hagen, 1984b). To see if there is also a relationship between vegetation and spine length, sites were scored as having abundant filamentous algae or abundant grasses. Some sites have roughly equal proportions, and others have neither in abundance but these were excluded because there were too few sites in either category to provide meaningful comparison. The results (table 3) show that spine length is greater for both species in the environments with grasses. Dense algae probably give better cover from predators than do grasses (Blouw and Hagen, 1984b), and predators are less abundant at sites with dense algae in any event (Blouw, 1982), so the differences in spine length are as expected if predation is influential.

(ii) Covariation with a new species of stickleback

Gasterosteus aculeatus (threespine stickleback) males usually develop red throats, blue irises, and cryptic bodies during the breeding season, but there is variation both within and among populations for breeding colours (Gilbertson, 1980; Hagen and Moodie, 1979; Hagen *et al.*, 1980; Moodie,

TABLE 3

Median tests for differences in standardised spine length of *Apeltes* and *Pungitius* among environments with and without predators (A), and with different types of vegetation (B)

	Predators present		Predators absent		$\chi^2(1)^a$	P
	< Median	> Median	< Median	> Median		
A. Spine Length						
<i>Apeltes</i>	1	6	20	15	—	<0.01
<i>Pungitius</i>	1	6	20	15	—	<0.01
	Abundant grasses		Abundant filamentous algae			
	< Median	> Median	< Median	> Median		
B. Spine Length						
<i>Apeltes</i>	3	10	13	6	4.66	<0.05
<i>Pungitius</i>	2	11	14	5	8.29	<0.01

^a For (A) probabilities were calculated by Fisher's exact test, for (B) by contingency χ^2 corrected for continuity.

1972a, b; Semler, 1971; personal observation). While sampling in Nova Scotia we discovered a new species of *Gasterosteus* that often coexists with *Apeltes* and *Pungitius*. When first discovered this stickleback was thought to be a colour morph of *G. aculeatus*, with which it also coexists at many sites. However subsequent investigations (Hagen, Blouw and Armstrong, in preparation) show that it is reproductively isolated and that it differs from *G. aculeatus* in its adult size, breeding ecology, behaviour, and most strikingly, in male reproductive colour. The dorsal and lateral surfaces of breeding males are iridescent white instead of cryptic green. This colouration is highly reflective, so males are very conspicuous and easily visible at a distance. We call this fish the "white" stickleback.

Two aspects of the environments in which the white stickleback is found are relevant here. First, it breeds only in dense filamentous algae (*Cladophora*), providing abundant and effective cover from predators. Second, we caught few predatory fishes at such sites. Hence the white stickleback breeds only where predation risk is low, as is suggested by the brilliant colouration itself.

Since the white stickleback breeds where predation risk is low, we predict that spine number for both *Apeltes* and *Pungitius* is lower where they coexist with the white stickleback. A quantitative test is complicated by uncertainty concerning where the white stickleback is absent. The breeding season is brief and highly synchronous within colonies, and breeding time differs among sites. Thus absence of the white stickleback from a particular site at one time is an unreliable indicator of whether it breeds at that site. To minimise this difficulty the white stickleback was scored absent only at sites that were sampled repeatedly but where it was not found. Note this is not an independent test; the white stickleback breeds only in dense filamentous algae and spine number is known to be lower at sites with this vegetation (Blouw and Hagen, 1984b). Nevertheless the test is worthwhile because an unexplored dimension of environmental heterogeneity (presence of the white stickleback) is involved, so the test is on a unique subset of sites with filamentous algae.

TABLE 4

Comparisons of Apeltes and Pungitius mean spine numbers among sites with and without the white stickleback by analysis of variance

Species	Mean spine number		Analysis of variance					
	Whites absent	Whites present	Source	SS	d.f.	MS	F	P
<i>Apeltes</i>	4.25	4.07	Among	0.7302	1	0.7302	30.8 ^a	<0.001
	(N = 37)	(N = 58)	Within	2.2052	93	0.0237		
<i>Pungitius</i>	9.78	9.27	Among	1.2570	1	1.2570	20.3	<0.001
	(N = 13)	(N = 8)	Within	1.1758	19	0.0619		

^a Variances are unequal by Levene's test, but recalculation using both the Welch and Brown-Forsythe statistics (Brown and Forsythe, 1974a, b) confirm the conclusion at the same level of significance.

Mean spine numbers of *Apeltes* and *Pungitius* are significantly lower where the white stickleback is present (table 4). This is another example of the non-random distribution of spines with respect to environment, providing further evidence that the polymorphisms are subject to selection by predators.

The breeding colours of *Pungitius* males are also extreme where they coexist with the white stickleback. At most sites in our study area *Pungitius* males develop a darkly pigmented spot between the pelvic spines, the pelvics are white, and the body remains olive or brown with dark blotches as illustrated in McKenzie and Keenleyside (1970). But where they coexist with the white stickleback, *Pungitius* males are often entirely jet black and the pelvic spines are a glossy blue-white. Thus they too are conspicuous in these environments with reduced predation risk.

4. DISCUSSION

The relationships among spine number, spine length, predators and vegetation are all parallel for *Apeltes* and *Pungitius*. This is strong evidence that selection is acting on the dorsal spines of both species. Since both species have more and longer spines where predators are present and where cover is reduced, and since predators are known to prey selectively on *Apeltes* (Blouw and Hagen, 1984c), the results are consistent with the hypothesis that predators are a common selective agent.

This conclusion demands a reinterpretation of McPhail's (1963) work on *Pungitius*. McPhail surveyed 132 samples for variation in four traits, including dorsal spine number and pelvic spine length, and hypothesised that present day patterns of variation in dorsal spines result from the dispersal of two forms of *Pungitius* that differentiated in separate glacial refugia during the Pleistocene era. He attributes local variation in the Maritime Provinces (and in some other areas) to gene flow between the two forms after post-glacial contact was re-established. The variation in pelvic spine length does not seem to parallel variation in dorsal spine number (but the method used to score spine length as a ratio of pectoral fin length seems questionable), and McPhail suggested that spine length

differences among sites may be induced by combinations of salinity and temperature.

Since spine number in *Pungitius* is subject to selection, we suggest the variation among populations reflects spatial heterogeneity in selective forces rather than historical events of differentiation, dispersal and gene flow in relation to glaciation. The hypothesis of spatially varying selection is testable, it accounts for the covariation between *Apeltes* and *Pungitius* in both spine number and spine length, and it accounts for the correlation between spine number and spine length within each species. As well as resolving issues regarding the observed differentiation in *Apeltes* and *Pungitius*, tests of hypotheses of spatially varying selection will contribute much needed empirical evidence to recent models of evolution in heterogeneous environments (Felsenstein, 1976; Hedrick *et al.*, 1976; Karlin, 1976, 1982; Powell and Taylor, 1979; Slatkin, 1978; Spieth, 1979), and to distinguishing between alternative hypotheses in biogeography (Endler, 1981, 1982a, b). If the geographic patterns produced by historical processes happen to coincide with present day geographical differences in selective regimes among sites, then the current patterns of variation may reflect historical events. Although we cannot disprove this, the likelihood of such coincidence seems small.

The correlation between the distribution of the white stickleback and spine number in *Apeltes* and *Pungitius* is also relevant in the context of evolution in heterogeneous environments because one component of environmental heterogeneity, filamentous algae, is identified as having a profound influence. The white stickleback breeds only in dense beds of filamentous alga (*Cladophora*), which provides exceptional cover from predators. And it is here that spine number for *Apeltes* and *Pungitius* is lower, spines are shorter, and the breeding colours of *Pungitius* are accentuated. It seems certain that the conspicuous colours of white *Gasterosteus* and black *Pungitius* males are for increased advertisement, and this may be explained by one or both of two hypotheses. First, sexual selection by female preference may favour enhancement of breeding colours in males (Endler, 1978, 1980, 1982c; Reznick and Endler, 1982; Semler, 1971) and the counterselection for crypsis exerted elsewhere by predators is reduced due to the abundant and effective cover. Second, males may be advertising to visually searching predators that they are unprofitable prey (Baker and Parker, 1979), unprofitable because of the excellent refuge provided by algae.

We are still left to explain why *Apeltes* and *Pungitius* have fewer and shorter spines at sites with dense filamentous algae. The three-spined morph is rare or absent throughout the range (Cox, 1923; Hildebrand and Schroeder, 1928; Krueger, 1961; Leim and Scott, 1966; Scott and Crossman, 1973), but in Denys Basin (Bras D'Or Lake, Nova Scotia) where there are few predators and abundant algae, it reaches remarkably high frequencies (Blouw and Hagen, 1984a). We have shown elsewhere (Blouw and Hagen, 1984b) that spine number in *Apeltes* is lower where it coexists with competitors, and we hypothesise that competition may select for lower spine number. Reimchen (1980) and Reist (1980a, b) suggest that invertebrate predators may be selective agents favouring reduced spines because many invertebrates grasp their prey, and spines may provide holdfasts. Thus it may be that reduced vulnerability to vertebrate predators at sites like those in Denys Basin allows increased response to competitors, invertebrate predators, or both.

The search for patterns of covariation within and among species has proven highly productive in this study, and it should be applied more frequently among species of sticklebacks. The group is conspicuously variable for many traits, various combinations of species coexist (particularly on the east coast of North America), they occur in diverse environments, and they have similar ecologies. The method of interspecific covariation is efficient because it provides evidence for two or more species simultaneously. Furthermore, when combined with detailed sampling, measures of environmental variation, and patterns of correlation among traits within species, it has the advantage of limiting the number of potential selective agents because the hypothesised agents must be consistent with all aspects of the patterns of covariation within and among species. A search for patterns of covariation among species may also reveal unsuspected ecological differences among them. For example, the covariation between *Apeltes* and *Pungitius* breaks down at the two sites in Louisbourg Harbour, and this suggests they differ in some aspect of their ecology or behaviour there. The discordance is of particular interest because it occurs at an intrusion for high spine number in *Apeltes*. Investigating the reasons for the discordance may suggest explanations for the intrusions found elsewhere (Blouw and Hagen, 1984a).

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