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Spatio-temporal microdifferentiation of water-strider (*Gerris*) populations

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SUMMARY

Spatial and temporal differentiation in *Gerris lacustris* and *G. odontogaster* (Heteroptera, Gerridae) were studied in restricted areas, where one population of each species was subdivided into several subpopulations. The aim of the study was to relate genetic population parameters to ecological population structure studied by mark-recapture methods in the same pond systems. Marked differentiation between the subpopulations and significant enzyme allele frequency changes between subsequent generations were found at several loci. It is suggested that non-selective forces are a sufficient explanation for the observed differences.

1. INTRODUCTION

Our previous studies on enzyme gene variation in water-striders (*Gerris*) have shown that the amount of gene diversity varies from one species to another. While searching for explanations for the observed patterns of this variation, we have come to the following conclusions: first, the amount of variation in different species correlates well with their population characteristics, the most abundant species being also the most variable ones (Varvio-Aho, 1980a), secondly, the degree of genic differentiation among local populations in a restricted area seems to be inversely related to the dispersion ability of the species. Thus *Gerris odontogaster* which has monomorphically long-winged overwintering generations shows less divergence between its local populations than the wing-polymorphic species *G. lacustris* and *G. lateralis* do. Third, although the enzyme loci studied differ greatly as regards the level of gene diversity, the relative differentiation shows no significant heterogeneity between the loci, suggesting that the loci are affected by similar evolutionary forces (Varvio-Aho, 1979; Varvio-Aho & Pamilo, 1979, 1980).

These previous observations do qualitatively agree with the hypothesis that patterns of genic variation are *mainly* affected by non-selective factors. Quantitative predictions are difficult, if not impossible to make.

If we want to determine the effects of stochastic processes on genetic variation, the best demonstration can be obtained by studying microgeographic patterns (e.g. Cavalli-Sforza & Bodmer, 1971). Such a study avoids the problems of population histories and possible geographic differences in selection. Another

approach for demonstrating the effects of genetic drift is to examine temporal changes in the gene pool. Allele frequencies always change due to random sampling of gametes in finite populations; the interest is in whether this is a sufficient explanation for all the changes.

In the present study we apply these two approaches in studying genetic structure of populations in two species of water-striders, *Gerris odontogaster* and *Gerris lacustris*. In each species, we have studied a population which is subdivided into a number of subpopulations inhabiting a set of closely located ponds. Our aim is to relate the genetic population parameters (spatial differentiation, temporal changes) to ecological population structure, studied by mark-recapture methods in the same pond-systems. Two aspects are involved in this analysis. First, we want to examine whether the observed patterns of variation can be explained by non-selective factors, the expected effects of which can be roughly estimated from the population parameters. Second, we get reference data to be compared with our previous observations on the magnitude of genic differentiation over larger areas.

2. MATERIAL AND METHODS

Microdifferentiation was studied in two species of water-striders, *Gerris odontogaster* (Zett.) and *G. lacustris* (L.) (Heteroptera, Gerridae) at seven polymorphic enzyme loci. The life cycles of the two species are as follows (Vepsäläinen, 1974). The adults overwinter on land, sometimes presumably rather far away from the ponds they live in, and they inhabit the ponds again in the late spring and early summer. In southern Finland this takes place mainly in May. After reproduction the old generation dies during the summer, and the new generation emerges from early July onwards. They are individuals developing diapause and overwintering, except a small fraction of the new generation in partially bivoltine *G. odontogaster*, which reproduces during the same summer. The overwintering generation of *G. odontogaster* consists solely of long-winged individuals, while in *G. lacustris* short-winged individuals, without flying ability, form a certain proportion of the population. The wing polymorphism in this species is genetically controlled and the percentage of short-winged animals varies from one population to another.

Gerris odontogaster was studied at Siikajärvi in Vihti, southern Finland (about 25 km west of the city of Helsinki). The population studied consisted of subpopulations inhabiting ponds on an open bog surrounded by coniferous forest. The open area was about 200 m wide and 1000 m long, containing some tens of ponds of varying sizes, the largest ponds having an area of 100 m^2. The ponds were mostly at least 2 m deep without risk of drying up, but the area of the water surface in the smallest and shallowest ponds markedly decreased during the summer. Most of the ponds were located in two rows at the eastern end of the bog and the study was carried out here within an area of 150 × 300 m. *G. odontogaster* lived in all ponds in the area. In the early summer *G. lacustris* was about as abundant as *G. odontogaster*, while only few individuals were seen in the late sum-

mer. The overwintered generation was sampled on 22, 25 May, 1, 19 and 20 June, and the offspring generation on 28 July, 18 August and 4 September.

Gerris lacustris was studied at Rusutjärvi in Tuusula (about 35 km north of Helsinki). The population inhabited shallow ponds on an open sandy field. There were four small ponds (5–10 m^2) separated by distances of a few meters. All the ponds contained water throughout the summer. The overwintered generation was sampled on 10, 16 and 24 June; the offspring generation was sampled on 15, 29 July, 19 August and 2 September.

The captured specimens were marked on the two first visits in all four ponds at Rusutjärvi (*lacustris*), and in five ponds at Siikajärvi (*odontogaster*) in order to estimate subpopulation sizes and rates of dispersion between the ponds. Population sizes were estimated by using either Bailey's triple catch method or the Lincoln index (e.g. Poole, 1974). The offspring individuals were marked during the first two visits in all ponds at Rusutjärvi, and in eight ponds at Siikajärvi.

The samples taken to the laboratory were analysed by starch gel electrophoresis, and stained for those enzymes known to be polymorphic in the two species (for the methods used, see Varvio-Aho & Pamilo, 1979).

3. RESULTS

(i) *Population parameters in* G. odontogaster

The overwintered generation was marked twice in two ponds (1 and 14) and the population size could be estimated by Bailey's triple catch method. This method gives an estimate of the number of individuals at the time of the second marking. Further, it allows an estimation of the loss of individuals between the first and the second marking, and the rate of increase in the population size from the second marking to the final catch. From this information we can estimate that the total number of individuals that lived in a given pond during the study period was about 1·5 times that estimated for a given day at the end of May.

In the ponds 6, 9 and 13 the population size was estimated by Lincoln index (including the so called Bailey's correction for small samples) separately for the two marking days. In the ponds where no markings were done, the population size was estimated roughly by sight. The estimated population sizes are given in Table 1.

There was a slight excess of females, their proportion in the pooled material being 55·7 % (393 out of 705) at the end of May, and 53·0 % (405 out of 764) in the latter half of June. When tested by the 2 × 2 chi-square test, the samples did not differ significantly from each other ($\chi^2 = 1{\cdot}10$, $P > 0{\cdot}10$), but the goodness-of-fit test showed the first ratio differing from the 1 : 1 ratio ($\chi^2 = 4{\cdot}65$, $0{\cdot}05 > P > 0{\cdot}01$) while the latter did not ($\chi^2 = 1{\cdot}38$, $P > 0{\cdot}10$).

From the total of 385 markings, we got 334 recaptures (one mark may occur one to three times in the recaptures). Of these, 13 (seven males and six females) were recaptured in an alien pond, making 4 % of all recaptures. As the recaptures

came partly from the same individuals marked and recaptured twice, we can roughly estimate that about 8 % of the *G. odontogaster* individuals visited at least two ponds in the study period. Dispersed individuals were met both at the end of May and later in June, and they were mainly captured in a pond next to the original one, the greatest observed distance being 50 m.

Table 1. *Population parameters in* Gerris odontogaster

Subpopulation	N_1	N_T	γ	β
1	167	220	0·040	0·008
14	111	189	0·069	0·013
6	81	~122		
9	32	~48		
13	41	~62		
2, 4, 18	Some tens			
8, 19, 20	Some hundreds			

N_1, population size at the end of May; N_T, total number of individuals between May 20 and June 20; γ, the loss rate due to deaths and emigration; β, the dilution rate due to immigration.

The offspring generation was marked over too great an interval to yield estimates of population sizes, as the emerged individuals start to move to their overwintering places during the summer. Of the 201 individuals marked in the first and 136 in the second visit, we got only 5 recaptures, suggesting that the population size in the offspring generation was several times greater than in the parental one.

(ii) *Population parameters in* G. lacustris

The triple catch method gave estimates for the overwintered population sizes of 65, 10, 67 and 23 for the ponds 1, 2, 3 and 4, respectively. The site tenacity of water-striders in the ponds was remarkable. Most of the individuals were marked (123 marked individuals) and could be subsequently recaptured in their own pond. Only one male was observed to have moved, from pond 1 to 3.

In the offspring generation the number of recaptures was again too small to yield any reliable estimates of their population sizes. Nevertheless, they were large compared to the overwintered generation. For instance, altogether 192 specimens were caught from pond 2, in which only seven females and three males were met in early summer.

(iii) *Genic variation and differentiation*

Taking the subpopulations of *G. odontogaster* separately, the average expected heterozygosity over the seven loci studied ranged from 0·212 to 0·334 in the overwintered and from 0·233 to 0·388 in the offspring generation, the means over subpopulations being 0·294 and 0·303, respectively (Table 2). These are of the same order of magnitude as previously found in other populations of the speices (Varvio-Aho, 1979; Varvio-Aho & Pamilo, 1980). The differences from the

previous results are that *Idh* was found to be more, and *Mdh* less, heterozygous than in southern Finnish populations in general, although *Mdh* was found to be completely monomorphic in northern Finnish populations.

The mean heterozygosity over the subpopulations in *G. lacustris* was 0·244 (range 0·222–0·253) in the overwintered and 0·289 (range 0·214–0·350) in the offspring generation (Table 3). These values are much lower than reported pre-

Table 2. *Frequencies of the commonest alleles, expected mean heterozygosities* (H), *and sample sizes* (n) *in* Gerris odontogaster

Sub-population	n	*Ao*	*Est–3*	*Est–4*	*Gpd*	*Idh*	*Mdh*	*Pgd*	$\bar{H}$
				Overwintered					
2	54	—	0·78	0·75	0·61	0·65	0·98	0·90	0·320
4	40	—	0·72	—	—	0·68	1·00	0·90	0·304
7	40	0·90	0·82	0·90	0·50	0·60	0·98	0·90	0·287
8	48	—	0·83	0·84	—	0·56	1·00	0·88	0·286
14	56	0·93	0·82	0·76	0·54	0·54	1·00	0·96	0·291
18	48	0·91	0·65	0·82	0·90	0·88	0·98	0·98	0·212
19	50	0·97	0·84	0·70	0·53	0·68	0·97	0·90	0·296
20	60	0·85	0·75	0·73	0·72	0·72	0·92	0·92	0·334
				Offspring					
2	56	0·84	0·63	0·56	0·68	0·80	0·86	0·91	0·388
4	36	—	0·83	—	0·64	0·67	0·94	0·94	0·307
7	30	—	0·77	0·87	0·77	0·80	0·97	0·97	0·233
8	46	0·90	0·89	0·61	0·73	0·54	0·98	0·94	0·296
14	54	0·91	0·70	0·65	0·80	0·67	1·00	1·00	0·285
18	50	0·92	0·76	0·60	0·70	0·64	0·96	1·00	0·308
19	54	—	0·80	0·73	0·61	0·74	0·87	0·93	0·326
20	48	0·92	0·71	0·59	0·78	0·67	1·00	0·92	0·314

viously from other populations. This reduction was largely due to two loci, *Ao* and *Pgd*, showing markedly less variation than detected earlier (Varvio-Aho & Pamilo, 1979, 1980).

The relative genic differentiation was measured by Wright's F_{ST} and Nei's (1973) G_{ST} statistics (Table 4). The former can be understood as correlation between alleles in the same subpopulation. The latter is based on the apportionment of genic variation into within- and between-subpopulation components, measuring the proportion of genic variation between the subpopulations from multiallelic data. The G_{ST}'s for the overwintered and offspring generations were 0·041 and 0·036 in *G. odontogaster* and 0·025 and 0·061 in *G. lacustris*. The corresponding F_{ST} values, when calculated for the commonest alleles and averaged over all loci, were 0·036 and 0·031 in *G. odontogaster* and 0·026 and 0·056 in *G. lacustris*. The values of F_{ST} varied somewhat from locus to locus, but the variance ratios (Lewontin & Krakauer, 1973) revealed no significant heterogeneities, the ratios of the observed variance of F_{ST} to that expected from $2\bar{F}^2/(s-1)$ being 1·30, 0·99,

0·99 and 0·68 for the four cases in the same order as given above (s is the number of subpopulations).

The absolute differences in the frequencies of the commonest alleles were examined by chi-square contingency tables. In *G. odontogaster* four loci (*Gpd*, *Idh*, *Est–3*, *Est–4*) could be tested. In the overwintered generation significant allele frequency differences between the subpopulations existed at *Gpd* ($\chi^2_{(5)} = 22{\cdot}5$, $P < 0{\cdot}001$) and at *Idh* ($\chi^2_{(7)} = 18{\cdot}4$, $P < 0{\cdot}05$). This heterogeneity disappeared in the offspring generation. In *G. lacustris* the allele frequency differences were tested at *Est–3* and *Est–6* in both generations, and at *Ao* and *Pgd* in the offspring generation only. No significant heterogeneity was observed in the overwintered

Table 3. *Frequencies of the commonest alleles, expected mean heterozygosities* (H), *and sample sizes* (n) *in* Gerris lacustris

Sub-population	n	*Ao*	*Est-3*	*Est-6*	*Gpd*	*Idh*	*Mdh*	*Pgd*	$\bar{H}$
				Overwintered					
1	40	0·95	0·50	0·75	1·00	1·00	—	—	0·232
2	20	0·95	0·63	0·90	0·95	0·90	0·80	0·80	0·249
3	36	0·94	0·50	0·83	0·94	0·97	0·92	—	0·222
4	18	0·83	0·50	0·83	0·95	1·00	0·85	0·85	0·253
				Offspring					
1	56	0·70	0·61	0·88	1·00	0·94	0·80	—	0·283
2	50	1·00	0·32	0·84	0·98	0·98	0·94	0·79	0·214
3	48	0·83	0·48	0·83	0·92	0·96	0·88	—	0·275
4	48	0·65	0·54	0·65	0·98	1·00	0·90	0·56	0·349

generation, but differences were found in the second generation at *Est–3* ($\chi^2_{(3)} = 9{\cdot}4$, $P < 0{\cdot}05$), *Est–6* ($\chi^2_{(3)} = 10{\cdot}0$, $P < 0{\cdot}05$) and *Ao* ($\chi^2_{(3)} = 12{\cdot}4$, $P < 0{\cdot}01$).

The differences between the subpopulations in *G. odontogaster* had a mosaic pattern, neighbouring ponds showing no genetic affinity to each other. Some significant correlations existed between the loci (calculated from the frequencies of the commonest alleles), the rank correlation coefficient being 0·821 both between *Gpd* and *Pgd* and between *Gpd* and *Idh* ($P < 0{\cdot}05$), and $-0{\cdot}943$ between *Gpd* and *Est–3* ($P < 0{\cdot}01$) in the overwintered generation. In the offspring generation the rank correlations between the loci varied from $-0{\cdot}381$ to 0·571, none of them being significantly different from zero. The allele frequencies in *G. lacustris* showed no clear correlations.

(iv) *Genotype frequencies*

Genotypic deviation from Hardy–Weinberg proportions was calculated for each locus from $F = 1 - H_{obs}/H_{exp}$, where H_{obs} refers to the observed frequency of heterozygotes, and H_{exp} is that expected from Levene's (1949) formula. Of 55 values of F calculated for the overwintered generation in *G. odontogaster*, 30 were positive, 18 negative and 7 values equalled zero. The ratio 30 : 18 does not differ

from a 1 : 1 expectation ($\chi^2_{(1)} = 3{\cdot}00$, $0{\cdot}05 < P < 0{\cdot}10$). In the offspring generation we found 25 positive and 25 negative F values, 5 values equalling zero. The mean $\bar{F}$ over the subpopulations and loci decreased from 0·10 to 0·06, respectively.

In *G. lacustris* the overwintered generation showed an excess of heterozygotes, there being 14 negative and 5 positive F values ($\chi^2_{(1)} = 4{\cdot}26$ when compared with the 1 : 1 expectation, $P < 0{\cdot}05$), 5 values equalling zero. In the offspring generation 11 values were positive, 7 negative and 3 zero. The mean $\bar{F}$ changed from −0·09 to 0·09.

Table 4. *Estimates of genic differentiation between subpopulations*

	G. odontogaster				*G. lacustris*			
	Overwintered		Offspring		Overwintered		Offspring	
	F_{ST}	G_{ST}	F_{ST}	G_{ST}	F_{ST}	G_{ST}	F_{ST}	G_{ST}
Ao	0·019	0·010	−0·001	0·004	0·042	0·059	0·145	0·118
Est-3	0·023	0·034	0·034	0·031	0·006	0·015	0·055	0·054
Est-4	0·026	0·036	0·046	0·065	—	—	—	—
Est-6	—	—	—	—	0·022	0·044	0·063	0·055
Gpd	0·090	0·062	0·023	0·022	0·017	0·016	0·043	0·027
Idh	0·047	0·046	0·030	0·030	0·071	0·056	0·021	0·028
Mdh	0·034	0·037	0·060	0·052	0·023	0·023	0·011	0·018
Pgd	0·016	0·017	0·025	0·028	0·001	0·004	0·114	0·056
Mean	0·036	0·041	0·031	0·036	0·026	0·025	0·064	0·061

The observed and expected genotype frequencies were also compared by chi square tests. For the tests we had to pool the rare genotypes together. None of the tests gave significant deviation between the observations and expectations.

(v) *Temporal changes in allele frequencies*

Temporal changes were analysed by comparing the allele frequencies in the successive generations of a given subpopulation. This was done either by 2 × 2 chi-square test or by Fisher's exact test, separately for each allele having a moderate frequency (moderate means that the expected frequency in the contingency table is five or more in both generations).

Of the 66 tests in *G. odontogaster* 14 revealed a significant difference at 5 % level, two of these being further significant at 1 % level. The change had a clear homogenizing effect at *Gpd*, the range of the frequencies of the commonest allele changing from 0·5–0·9 to 0·61–0·80, and directional effect at *Est–4*. The frequency of $Est\text{-}4^{100}$ decreased in six of seven subpopulations, the mean frequency over all subpopulations changing from 0·79 to 0·66, and being compensated by an increase in frequencies of several rare alleles. Ten of the significant changes took place at these two loci.

In *G. lacustris* two tests out of 27 gave a significant difference between the successive generations, namely at *Ao* in the subpopulation 1 and at *Est–3* in 2.

One way to estimate the relative roles of selective and non-selective forces in temporal changes is the use of Wright's (1943) expression, which relates the standardized gene frequency variance (F_{ST}) to the effective population number (N_e) and time. The homogeneity of F_{ST} values over the loci can be tested by the variance ratio $s^2_{F_{ST}}/2\bar{F}^2_{ST}$, where the denominator gives the approximate expected variance of F_{ST} values (Lewontin & Krakauer, 1973). For other details of the application of the method, see Varvio-Aho (1980*b*) and Pamilo & Varvio-Aho (1980).

Table 5. *Analysis of temporal allele frequency changes*

Sub-population	k	$\bar{F}_{ST}$	$1/\bar{F}_{ST}$	$s^2_{F_{ST}}$	σ^2	Variance ratio
			G. odontogaster			
2	11	0·0598	16·8	0·0043	0·0071	0·61
4	4	0·0205	49·0	0·0008	0·0008	—
7	9	0·1020	10·0	0·0106	0·0208	0·51
8	6	0·0806	12·4	0·0249	0·0130	—
14	10	0·1157	8·6	0·0343	0·0268	1·28
18	8	0·1991	5·0	0·0433	0·0793	0·55
19	11	0·0406	24·6	0·0025	0·0033	0·76
20	9	0·0817	12·2	0·0093	0·0133	0·70
			G. lacustris			
1	7	0·2333	4·2	0·2125	0·1089	—
2	8	0·1122	9·2	0·0169	0·0252	0·67
3	7	0·0421	23·8	0·0073	0·0035	—
4	10	0·1599	6·2	0·0391	0·0511	0·80

k, the number of alleles involved; $\bar{F}_{ST}$, the mean standardized variance of the temporal allele frequency change ($F_{ST} = (\Delta q)^2/q_0(1-q_0)$): $s^2_{F_{ST}}$ the observed variance of F_{ST} values over loci; σ^2, the expected variance of F_{ST} from $2F^2_{ST}$.

F_{ST} was calculated for alleles with a frequency within the limits 0·05–0·95 in both generations. Table 5 gives these values, averaged over all alleles used, the observed and expected variances of F_{ST}, and the variance ratio. None of the variance ratios was statistically significantly different from 1·0 with 1, ∞ degrees of freedom. Variance ratios are not given for those cases which include less than eight F_{ST} values.

4. DISCUSSION

Our findings of microgeographical differences between *Gerris* subpopulations concur with the previous studies in other animals suggesting great genetic heterogeneity in small areas. Area effects in *Cepaea* colour polymorphism are a classical example (see Jones, Leith & Rawlings, 1977, for a review) and they may be associated with other genetic differences (Johnson, 1976). The studies on microgeographic variation in allozyme frequencies are not numerous, but they suggest that microspatial differentiation is a rule rather than an exception (e.g. Selander &

Kaufman, 1975; Taylor & Powell, 1977; Richmond, 1978). The situation found here in *Gerris* resembles that in house mice, in which the mosaic allele frequency distribution within a small area looks like a variation pattern in large geographic areas (Selander, 1970).

Microgeographic differentiation can result from at least four factors: genetic drift, natural selection varying with the location of an individual in the environment, non-uniform dispersal of different genotypes, and habitat selection which is genetically determined (Richmond, 1978). In the present study we examined subpopulations in such a restricted and homogeneous area that it is difficult to imagine differing selection pressures within the study area. The effects of history should also be minimized in such a scale, but this was not so clear with *G. odontogaster*. In the overwintered generation at Siikajärvi, we found *G. lacustris* almost as abundant as *G. odontogaster* but only a few individuals were detected in the offspring generation. It may be that there is a replacement of the dominant species taking place in the study area; such changes may occur in a short time (see Vepsäläinen & Järvinen, 1974, for an example).

Differentiation due to genetic sampling processes, i.e. genetic drift, is opposed by gene flow between the subpopulations. In water-striders this may take place in two ways: the overwintered individuals do not return to their original pond, or the individuals may disperse from one pond to another during the breeding season. In *G. lacustris* at Rusutjärvi the subpopulations were separated by only a few meters, and it is reasonable to assume that considerable mixing of individuals occurs when they return to the ponds in spring. The observed homogeneity in the overwintered generation agrees with this. In *G. odontogaster* the overwintered generation showed remarkable differentiation between subpopulations at several loci, suggesting at least partial isolation of the ponds. The subpopulations studied in *G. odontogaster* were separated by some tens of meters, which may be enough to suppress efficient mixing in spring. However, the dispersion rate estimated to be 4–8 % in early summer should be large enough to smooth out any differentiation in the long run. Theoretical studies have shown that extremely low gene flow is sufficient to homogenize the populations (e.g. Nei & Feldman, 1972). The problem is, has there been enough time? As discussed above, it may be that *G. odontogaster* was a newcomer in the area.

The genotype frequencies of the overwintered generation in both species agreed with the Hardy–Weinberg expectations. However, there was a slight tendency towards an excess of homozygotes in *G. odontogaster*, and an excess of heterozygotes in *G. lacustris*, which were smoothed out in the offspring generation. Such tendencies might arise as a consequence of mixing of slightly differentiated gene pools in the formation of the spring populations. This seems to contradict the assumption of pond fidelity in *G. odontogaster*, reflected by conspicuous differentiation in the overwintered subpopulations, but, as noted, the deviation from the expected genotype frequencies was not significant in this species.

When moving from the overwintered to the offspring generation, average genic differentiation remained by and large unchanged in *G. odontogaster* but greatly

increased in *G. lacustris*. The latter change is understandable as the subpopulations were quite small. As the subpopulation sizes manifoldly increased in the offspring generation, a sampling error in favour of the offspring of a couple of females could result in marked changes in allele frequencies and increased differentiation between the ponds.

The constancy of average G_{ST} and F_{ST} in *G. odontogaster* includes changes at various loci, opposing each other's effects. The focus should thus be on whether the allele frequency changes at specific loci can be explained by stochastic processes or not. This was done by calculating the F_{ST} values on the basis of temporal changes. Homogeneity of these values over the alleles studied indicates that the alleles at different loci were affected by similar agents, most probably by genetic sampling processes, and by our own sampling error. Applying the absolute value of F_{ST} in estimating the causes of allele frequency changes can be based on the fact that in a population with effective size N_e the expected change gives $\overline{F}_{ST} = 1/2N_e$. This holds for the population. But when F_{ST} is estimated on the basis of samples from successive generations, it is no more related to the population size, but rather to the sample size used, $1/\overline{F}_{ST}$ approximately equalling the number of individuals sampled (Pamilo & Varvio-Aho, 1980). This was specially detected by computer simulations applying population sizes and sample sizes of the same order of magnitude as in the present study.

In our results, the estimates of $1/\overline{F}_{ST}$ agreed with that expected in several subpopulations, but they often fell below the expected value. This suggests that the allele frequencies in the populations have changed more than expected on the basis of drift alone. This could result from several reasons which are not necessarily mutually exclusive. First, the homogeneity of the values are due to similar selection at each locus studied. This seems quite improbable. Second, although the variance test showed homogeneity of the changes, we have to be careful when making definite conclusions as the test is not very powerful. There still remains a possibility that moderately large changes at *Gpd* and *Est-4* in *G. odontogaster*, which now bias the mean $\overline{F}_{ST}$'s, are associated with some type of selection at these or linked loci. The third explanation, and the one we favour, is that the small estimates of $1/\overline{F}_{ST}$ are due to the offspring developing in cohorts. The females lay eggs in batches. The sampling in the population need not be simple sampling of individuals but a biased sampling of sibling groups, as the food and weather conditions and cannibalism during the larval period make the conditions for development differ between sibling groups (Varvio-Aho *et al.* 1979). This may increase the variance of allele frequency changes and result in small values of $1/\overline{F}_{ST}$. Another feature associated with the existence of sibling groups is that our samples taken at the very end of the summer do not represent the whole offspring generation, but may consist of a small number of sibling groups.

If we have been observing selective allele frequency changes, we have to invoke many different selection pressures, working in opposite directions even in adjacent ponds, as only a minor part of the changes were systematic. In conclusion, if selection is involved, it must be something that concerns many loci

simultaneously. To analyse such a situation is impossible at the moment, as the theoretical framework is missing.

The demonstration of the existence of marked differentiation on a micro-scale, and the occurrence of various allele frequency changes on the same scale, which are of the same order of magnitude as in large geographical areas, support our earlier conclusions on the causes of differentiation and allele frequency changes.

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